THE CYTOLOGY OF MYELIN PRODUCTION

IN THE

CENTRAL NERVOUS SYSTEM:

A REVIEW OF THE PROBLEM

WITH SOME PERSONAL HISTOLOGICAL OBSERVATIONS

Thesis

submitted for the degree of M.D. in the University of Edinburgh

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PREFACE

The object of this research is to discover if any specific cell is responsible for the production of myelin in the central nervous system. My interest in this problem began when I was studying the demyelinating diseases, for although there is an extensive literature on experimental demyelination and its histological features, I could find little information about the cytology of normal myelin production in the central nervous system (in contrast to the peripheral nervous system).

We know that myelin is a mixture of fatty substances forming a sheath around certain nerve axons, but the histological methods used to demonstrate the fully developed sheath have only occasionally been used in attempts to show which cells of the central nervous system are concerned with myelin production. I therefore decided to use histochemical methods to study the earliest signs of myelin deposition, hoping that a critical use of methods for the demonstration of lipids might help to answer the problem of the cytological mechanisms of myelin production.

The first part of the thesis consists of reviews of the present state of our knowledge of myelin production in the central nervous system, and of biochemical work on the composition of myelin. This chapter is introduced because

I consider that it is essential to define the chemical composition of myelin as accurately as possible before attempting to use histochemical methods.

The second part is concerned with my own investigations of selected regions of human and animal central nervous system during development. Sections of nerve tracts were examined before and during myelination, to see if any cellular activity could be correlated with myelination. My deductions are offered as a contribution to our knowledge of myelin production. In some ways, the problems of myelin can be compared with the problems of collagen, on which biochemists, physicists, anatomists, and clinicians have all worked together; and wherever possible, I have correlated my own histological results with those of other workers examining the same problem with different methods.

THE FORMATION OF MYELIN IN THE CENTRAL NERVOUS SYSTEM

The cells of the central nervous system are of two main varieties, the highly specialised nerve cells and their processes, forming the parenchyma, and the less highly specialised neuroglial cells and processes, forming the supporting tissue. It is also probable that there is a ground substance which, in addition to functioning as a matrix, may be important in nerve metabolism (Hess 1953). A remarkable morphological feature of the nerve cell body is its continuity with a long process, the axon. Fully developed axons are surrounded by a mixture of fatty substances, the myelin sheath, which in the fresh state gives the characteristic white appearance to parts of the central nervous system. The present investigation is an attempt to discover which cells in the central nervous system are mainly responsible for the production of myelin. This chapter reviews the conclusions of other workers on this subject.

Histological examinations

In making comparisons of the results of histological work, the varying species of animals used must be taken into account. Table 1 enumerates the species examined by previous workers.

TABLE 1

Animal species examined by previous workers.

Summary of conclusions.

Virchow	1867	Man	CNS	fat	cells	pathological
Boll	1874	Chick	"	"	"	physiological
Jastrowitz	1872	Man	"	"	"	physiological
Wlassak	1898	Chick Rabbit Mouse	"	"	"	physiological
Sattler	1915	Man Rabbit	"	"	"	physiological
Wohlwill	1921	Man	"	"	"	physiological
Schwarz	1924	Man	"	"	"	pathological
Berberich	1926	Man	"	"	"	pathological
Morrison	1931	Pig Cat	"	"	"	physiological
Linell & Tom	1931	White rat	"	"	"	physiological
Langworthy	1934	Man	"	"	"	physiological
Alpers and Haymaker	1934	Man	"	"	"	physiological
Roback and Scherer	1935	Man	"	"	"	pathological
Tuthill	1938	Man	"	"	"	physiological

The work now reviewed had not always had the specific aim of deciding what cells in the central nervous system are responsible for myelin production. The earliest paper with relevant material was by Virchow (1867). He described the pathological anatomy of the infant brain, with particular reference to the occurrence of fat-laden cells. In the brains of infants dying suddenly he found enlarged neuroglial cells filled with fat globules. The globules eventually extruded the glial cell nucleus entirely, assuming a spherical form, without a cell membrane. These fatty changes were mainly present in the white matter.

Virchow considered that the glial cells were pathological, and that the fatty changes were probably due to a myelitis or encephalitis. An interesting feature of these observations was that Virchow was satisfied that he could trace various stages of neuroglial fatty metamorphosis, from intracellular to extracellular fat. At the time he wrote, the various types of neuroglia had not been described, so we do not know whether the fat Virchow described was present entirely in microglia or not. (Virchow himself was the first to describe neuroglia as a distinct entity, according to Weigert - 1895).

The lack of accurate knowledge of central nervous system morphology, and the natural preoccupation of anatomists with the search for techniques to elucidate the detailed cytology of the CNS probably explains why so few attempts

have been made to use the available fat staining methods to study myelination. Nevertheless, several early workers used histochemical methods to study myelin formation.

Boll (1874) studied the development of the white matter in the chick nervous system, and decided that the myelin sheath was laid down by the confluence of fatty droplets which appeared between the naked axons. He also attempted to study the process by examining cells on a warm microscope stage, concluding that certain amoeboid cells were responsible for depositing the myelin fat.

A somewhat similar interpretation of the presence of such cells containing fat, in the central nervous system of human infants, was advanced by Jastrowitz (1872). His view was thus antagonistic to that of Virchow.

In the few years before the turn of the century, a considerable amount of biochemical work was in progress, to determine the chemical composition of the brain. This appeared to have stimulated Wlassak who, in 1898, produced an extremely interesting paper on the origin of myelin. One criticism of the work, which could also be levelled against many subsequent papers, was that too much reliance was placed on the specificity of the particular staining techniques used. Wlassak used Weigert's haematoxylin method to demonstrate "protagon", and the secondary blackening reaction with osmium to stain lecithin. (see page 44) We know today that "protagon" is not a pure substance, but a mixture of substances.

Our knowledge of the chemical composition of the brain has thus advanced, but unfortunately we still do not know which component is demonstrated by the Weigert method for myelin. Wlassak concluded that lecithin and neutral fat were the first substances to be deposited during myelination, and that "protagon" appeared later. We shall see (chapter 2) that it is doubtful if lecithin is a component of the sheath. He decided that embryonic glial cells had a metabolic as well as a protective function, carrying certain substances from the blood to the nerve fibre for incorporation into the myelin sheath.

Thus at the end of the century opinions were divided. On the one hand, pathologists were uncertain about the interpretation of the fatty changes in infant brains; on the other hand, workers with animal material tended to interpret such changes in developing nervous system as being concerned with the formation of myelin.

Sattler (1915) studied the direction of myelination in the optic nerve, and also added some observations on the formation of myelin. He too thought that lecithin granules were deposited in the vicinity of developing nerve fibres, and then became confluent during the formation of the sheath.

The pathological controversy was revived by Wohlwill (1921). He examined the brains of 77 infants, and concluded that the fat granules present were physiological, and

involved in myelinogenesis. Schwarz (1924) disagreed; he studied 100 brains and concluded that the presence of fat was in some way associated with trauma. Berberich (1926) continued the argument. I found his paper interesting because he made an examination of the olivary region, an area I have examined myself. In tracing the development of myelinated fibres from the olive, Berberich found only a few fibres present at the 34 cm. stage (39 week foetus). Here, however, my experience differs. In sections studied at various stages of development of the olivary region, I have seen numerous fibres entering or leaving the olive long before term. Lucas Keene and Hewer (1931) also found olivo-cerebellar fibres myelinating at 22 weeks. Romanes (1947) pointed out however that there was a considerable variation in the findings of different authors on this subject of the time of myelination in the various tracts of the central nervous system. I was not directly concerned with this aspect of myelination, but Berberich made the point that normal myelination proceeded in all parts of the medulla without the presence of fat cells; indeed, when these were present he considered that they were of pathological significance.

It is convenient at this point to break the chronological sequence of this review to pass to an important paper by Roback and Scherer (1935). These workers studied primarily the microscopic anatomy of the brain of newborn

infants, with special reference to the development of glial tissue. Embryologists and experimental cytologists had hitherto confined their attention to earlier stages of development. Roback and Scherer considered their particular study on infant brain was necessary because, at that time, there was no adequate description available of that period in development. This observation seems justified, and it was therefore not surprising that work on the cytological mechanisms of myelin production in human brain had not advanced, since purely morphological descriptions of the infant brain were so inadequate.

Their paper included some remarks on the interpretation of fat granules in the brains of infants. Roback and Scherer said that the problem was a difficult one, but they were convinced that stainable fat in the brains they examined was not connected with myelin formation. They believed that myelination proceeded under the influence of the glial cells, and they used the term "myelinisationgliosis" to describe this process. This was an interesting viewpoint, because it seemed that if such a conclusion were valid, then it ought to have been possible to advance further, and discover <u>how</u> the glial cells influenced myelination.

Further attempts to answer this problem by histological methods were made by Morrison (1931), Linell and Tom (1931), and Alpers and Haymaker (1934).

Morrison discussed the role of oligodendroglia in the genesis of myelin. He examined pig and cat embryos with the Weigert-Pal technique for myelin, and Hortega's silver carbonate method for oligodendroglia. It is not clear from his account how many embryos he studied. He described his findings in 10 cm., 11 cm., and 16 cm. pig embryos, and one adult cat. He found that numerous cells with an affinity for the myelin stain were present during the myelination of axons. These cells disappeared as myelination was completed. The cells he described as bearing myelin had a very scanty cytoplasm, and their processes were difficult to impregnate with the silver method. It is pertinent to compare Morrison's interpretation of these cells with the views of Roback and Scherer (op.cit.) on neuroglia and myelin formation. Morrison thought that the cells present in large numbers during myelination were immature oligodendroglia. Roback and Scherer thought that myelination was effected through the influence of incompletely differentiated oligodendroglia, which became fully differentiated only when myelination was completed.

The part played by oligodengroglia in the formation of myelin was also studied by Linell and Tom (1931). They examined the brains of white rats, and found morphological changes which were suggestive of the participation of these cells in myelin formation. Unfortunately, the only available

account of their work was in summary form which made its assessment difficult, for there were no details of techniques or of the histological findings.

Alpers and Haymaker (1934) added further observations on human foetal material, ranging from the 5th. foetal month to term. During the 5th. to 9th. month, they found that there were fat globules in the vascular feet of the astrocytes; from the 6th. to 9th. month fat droplets were found in the oligodendroglia; and from the 7th. month onwards, microglia contained fat. The authors used sudan IV and the Marchi reaction to demonstrate fat. With Weigert's myelin method they found blue spherical bodies in the cytoplasm of the astrocytes at the 9th. month.

Alpers and Haymaker did not specify which brain areas they examined. This is an important point, because naturally there were different activities in the same locality at different times. It is by no means certain that the spherical bodies in the astrocytes were fat or related to myelination simply by virtue of their staining with Weigert's method. From these results no clear picture of the part played by neuroglia in the formation of myelin could be formed.

Langworthy (1933) also found that in many immature brains numerous cells were stained with the myelin stain at the time of the first appearance of myelin. He did not specify his staining methods, nor pursue these observations further.

Another interpretation of the fat granules present in

human infant brains was advanced by Tuthill (1938). He related the fat deposits to poor capillary circulation, and considered that they were related to waste products of myelin metabolism.

Repair, regeneration, and tissue culture of the nervous system.

It is not my purpose to review the large amount of work which has been done on repair, regeneration, and tissue culture of the central nervous system. I wish only to mention some aspects of these methods of inquiry which have a bearing on the present investigation.

Workers who have investigated the effects of experimental surgical injury on the central nervous system have not reached any agreement about the possibility of repair and regeneration. Sugar and Gerard (1940) claimed to have demonstrated regeneration in the transected spinal cord of rats. They said many new nerve fibres were found in the scarred operation area. Unfortunately, they did not investigate their material with myelin staining methods. These findings have been disputed by subsequent workers. (Brown and McCouch, 1947; Davidoff and Ransohoff, 1948: Barnard and Carpenter, 1950). Although they agreed on the absence of repair, they disagreed about the role played by glial proliferation in its prevention. Brown and McCouch thought there was evidence of growth of severed axons, but complete regeneration was prevented by scar formation. None of these investigators studied their material with fat stains, which might have provided interesting histochemical

details about the glial proliferation.

No evidence about the cytology of myelin production has been obtained from the study of experimental injury and repair in the central nervous system. It seems, however, that if myelin stains as well as silver impregnations were to be used in future investigations, useful information might be forthcoming.

The morphology of cells of the central nervous system and many aspects of their behaviour have been studied in tissue culture (Lumsden and Pomerat, 1951: Hogue,1947,1953), but no evidence has yet been published about the lipid metabolism of these cells. Pomerat has suggested (1955) that neuropathological material as seen in tissue culture could be studied to great profit with cytochemical techniques.

The most important evidence in the study of the origin of the myelin in the central nervous system has therefore been provided by histological examination of foetal and embryonic material. Evidence will probably be provided in the future by more specialised techniques. In the peripheral nervous system, the electron microscope is being used to study myelination, and earlier views on the importance of the Schwann cells in the production of the sheath have been substantiated (Geren, 1954: Robertson, 1955). Electron microscope studies on developing central nervous system will, no doubt, also be made.

THE CHEMICAL CONSTITUTION OF MYELIN

Detailed chemical study of myelin is handicapped by the difficulty of obtaining it separated from other tissue. Fats, carbohydrates and proteins all enter into its composition. The lipids are responsible for many of the physical characteristics of white matter, of which they form approximately 60 per cent of the dry weight (Brante 1949). They are probably the most important constituents, but the proteins and carbohydrates with which they may be bound must always be considered when assessing function. It will also be seen from the classification of the lipids which follows, that inorganic constituents are also present in the myelin sheath.

Before reviewing previous work on the nature and composition of myelin, a short statement of the nomenclature of the lipids is necessary. This is not a classification of the lipids, which would be outside the scope of this work, but defines the terms used in this thesis.

As a group of substances, the lipids are in general esterlike compounds of fatty acids with alcohols, and sometimes with other substances. They may also be defined as a group because of their solubility in such substances as ether, chloroform, xylol and alcohol. Deuel (1951) adds the qualification that they must be utilisable by the animal organism, which excludes the mineral oil derivatives.

(1) Simple Lipids. The neutral fats are glycerol esters of

the fatty acids (such as palmitic, stearic, oleic). Each molecule of glycerol, a trihydroxy alcohol (trihydroxy propane) can combine with three fatty acid molecules, and the resulting triglycerides form a large proportion of the naturally occurring fats. Neutral fats, together with the waxes - which are esters of alcohols other than glycerol - form the group of simple lipids.

(2) Compound Lipids. If the fatty acid combines with other groups in addition to the alcohol, then a compound lipid is formed. The lipids of importance in the study of myelin are in this group. They can be more easily classified in tabular form.

A Phospholipids, Phosphatides. These all contain a phosphoric acid group.

1 Lecithin. Hydrolysis products: glycerol, fatty acids, phosphoric acid, choline.

2 Phosphatidyl serine. Hydrolysis products: glycerol, fatty acids, phosphoric acid, serine. Cations K.Na.

3 Phosphatidyl ethanolamine. Hydrolysis products: glycerol, fatty acids, phosphoric acid, ethanolamine (colamine).

4 Phosphatidyl inositol: brain diphosphoinositide. Hydrolysis products: glycerol, fatty acids, phosphoric acid, inositol. Cations Ca.Mg.

5 Cephalin. This is probably a mixture of the last three phosphatides above (Folch, 1942).

6 Sphingomyelin. This compound does not yield glycerol on hydrolysis. There are fatty acids, phosphoric acid, choline, and the nitrogenous base sphingosine.

7 Plasmalogens. These are compounds of higher fatty aldehyde acetals with ethanolamine (colamine) glycerophosphate.

B Carbohydrate-containing lipids. These do not yield glycerol on hydrolysis.

Cerebrosides. In this group a carbohydrate is an essential part of the molecule. The carbohydrate can be either galactose or glucose and the other hydrolysis products are a fatty acid and sphingosine.

(3) Derived lipids. Hydrolysis of simple and compound lipids may yield substances with characteristic lipid solubilities. These are derived lipids, and included in the group are fatty acids, some sterols, and vitamins A, D and E. Of the sterols cholesterol is an important constituent of the central nervous system, forming 14 per cent of the dry weight of the white matter, and 6 per cent of the grey matter. In the brain it occurs almost always in an unesterified form, whereas blood serum contains both free cholesterol and its esters.

The methods used to determine the composition of myelin have been indirect, using comparisons between chemical analyses of white and grey matter of adult brains, and between brain areas before and after myelination. In the peripheral

nervous system, the study of the chemical products of Wallerian degeneration has been another method. In addition to direct chemical analysis of nervous tissue, histochemical studies have also been made.

It has long been relaised that there is a significant difference in the lipid composition of white and grey matter, one of the first workers to demonstrate this being Thudichum (1884). The methods of the older chemists were inexact and required the use of large tissue samples. Species differences must also be remembered when comparing the results of different workers on lipid composition of the tissues. The use of newer micro-methods and the discovery in recent years of new lipids necessitate a re-estimation of the distribution of brain lipids. Estimations of the lipid content of whole brain do not help in deciding which lipids constitute the myelin sheath, but the high concentration of cholesterol, cerebrosides, and sphingomyelin (beef brain,Kaucher,Galbraith, Button and Williams,1943) and the fact that the normal human brain contains about 5-6 times the amount of sphingomyelin in any other organ should be remembered (Thannhauser, Benotti, and Reinstein, 1939). Johnson McNab and Rossiter (1948a) examined the lipid contents of normal brain and estimated free and total cholesterol, cerebrosides and phospholipids, in whole brain and in white and grey matter. Their results showed that the white matter contained a higher concentration of cholesterol, phospholipids and cerebrosides than did the

grey matter. An interesting fact was that if the proportions of sphingomyelin, lecithin and cephalin in the total phospholipid were considered, the spingomyelin was seen to be distributed mainly in the white matter, that is, in the axons and their myelin sheaths. Johnson et al,(1948a) thought that their results indicated that cholesterol, sphingomyelin and cerebrosides formed the chemical basis of myelin. Their results also showed that the grey matter contained considerable amounts of lipid; for example, the amount of cholesterol in grey matter was greater than in any other tissue, except possibly the adrenals.

No conclusions could be drawn from these results about the distribution of lipids between myelin, axon, and glial cells of white matter. For whole brain estimations Johnson et al (1948a) used guinea pig, cat and rabbit, and for white and grey matter they used cat, dog, beaver and man. In the account of their methods, they did not say how or from what areas they obtained the samples (1-2 grams) of the white and grey matter, and it was difficult to separate grey and white matter completely. The differences they found in their lipid constituents were so great that these objections are only of minor importance.

The fact that axons may have contained a good deal of lipid was, however, important. Johnson, McNab and Rossiter (1949) attempted to discover which lipids appear during myelination of fibre tracts, by comparing the concentrations

of lipids in the brain of human infants and adults. They examined five infant and five adult brains. They did not say from which areas they took the infant "white" matter nor whether they compared this "white" matter with topographically similar areas in the adult. The infants' ages ranged from two months premature to full term. They concluded that those areas of infant brain which could be assumed to become the white matter of adult brain corresponded in lipid content to adult grey matter. The lipids which increased during myelination were cholesterol, sphingomyelin and cerebrosides. The results are extremely interesting and confirm similar work by others.

Brante (1949) studied the distribution of lipids in the nervous system, using micro-methods very like those of Johnson et al (1948;1949). He concluded that in man the axon lipids were probably lecithins and colamine-containing cephalins, whereas the myelin sheath probably contained cholesterol, cerebrosides, sphingomyelin and phosphatidyl serine. He found the lipid composition of different cortical areas did not differ much; but there was a relatively higher cerebroside and cholesterol content in the thalamus, which was in agreement with the probable admixture of myelinated fibres in this area. His results on the lipid distribution in various parts of the human nervous system during development were also interesting. He considered three periods: 1) The time of axon formation without myelination; approximately from the second to the

sixth month of foetal life. 2) The period of myelination, from the sixth month of foetal life to approximately one year after birth. 3) Advanced age, when some lipid changes became apparent histologically. The first two periods were important for the present study. Brante found that the sphingomyelin content of foetal brains was almost negligible, but that it increased after birth, during the period of myelination. Cerebrosides and cholesterol almost doubled their quantity if infant cortical and white matter was compared with "outer" and "inner" layers of foetal brain. Another way of expressing this was to say that the foetal "inner" layer corresponded approximately to the infant grey matter.

One criticism may be made of Brante's work. It is difficult to obtain sufficient "normal" human material to reduce errors due to individual variation, but the inclusion of brains from patients dying of such varying causes as coeliac disease, barbituric acid poisoning, ether narcosis, uraemia and acute yellow atrophy of the liver is open to objection.

Schmidt, Benotti, Herschmann and Thannhauser (1946) showed that beef brain white matter contained approximately seven times more sphingomyelin than the grey matter. It was reasonable to conclude that the great difference was due to the presence of myelinated fibres. Williams (1945) estimated the lipids in rat brain in three groups of animals of age 15,

45 and 70 days. The cerebrosides and free cholesterol were approximately doubled from 15 to 70 days. The figures for sphingomyelin content did not compare with those of Johnson et al, nor with Brante's, for the increase was from 3.9 per cent of dry weight to 4.2 per cent. A method of sphingomyelin estimation was used which has since been criticised as inaccurate.

Schuwirth (1943) compared the lipid content of the spinal cord and brain, and found that the sphingomyelin concentration was higher in the cord than in the brain. This may have been because of the relatively higher amount of grey matter in the brain. The same worker (1940) examined the lipid content of whole human brain during development and concluded that the lipids which increased after birth were sphingomyelin, cerebroside and cholesterol. The figures he gave did not substantiate the text statement about cholesterol. He examined 38 brains: 34 were of foetuses and new born babies, 2 were children 13-14 months, and 2 adults of 64 and 78 years. The results would have been more interesting if the number of brains in each group had been approximately equal. Schuwirth's results were also not strictly comparable with those of Johnson and others, because he used different techniques. An example of the difference in results was that Schuwirth found no sphingomyelin in whole brains of foetuses and new-born infants. Histological examinations have shown that myelination has advanced in the brain as a

whole at the time of birth, and it is reasonable to suppose that the newer methods of Johnson, and others, gave more accurate results.

Randall (1938) examined 23 adult human brains, of ages from 28 to 82 years, and showed some important differences between the white and grey matter. The grey areas gave lower values for total lipids than the white areas; the cholesterol content of the grey areas was approximately half that of the white; but there was more phosphorus in the grey areas than the white. This last difference Randall attributed to the higher cerebroside content of the white matter, as cerebrosides contained no phosphorus. He explained that he took great care to obtain grey matter free from white. Only seven of the brains were regarded as normal. Of the others, twelve had serious mental disease and four had advanced arterio-sclerosis. These conditions could be associated with abnormal fat metabolism in the brain.

Randall seems to have been the first to examine the brain lipids in detail after the pioneer work of Lorrain Smith and Mair (1911-13), Koch and Koch (1913) and MacArthur and Doisy (1918-19).

Lorrain Smith and Mair (1912-13) were the first to study developmental changes in human white and grey matter. They examined formalin fixed material. Cerebrosides increased from 1.2 per cent in the white matter of a full-term stillborn, to 9.9 per cent in a 14-month-old child. Cholesterol

increased from 7.0 per cent to 13.0 per cent. During this time there was no comparable change in the grey matter concentrations of these lipids.

Koch and Koch (1913) studied the lipid changes in the albino rat brain during development. They showed that although phosphatides increased during the medullation period, these lipids were already present in large amounts before medullation, and some were therefore in the axons. They did not consider individual phosphatides. Lipids which appeared and increased during medullation were the cerebrosides.

Human brains were examined by MacArthur and Doisy (1918-19) who also concluded that the cerebrosides were directly related to myelin sheath formation. They stated that they had only used six brains of ages three months foetus to twenty-four months child, and four adult brains, and that disease may have caused chemical alterations.

No distinction can yet be made between myelin of the central nervous system and of the peripheral nerves. The possibility of a difference in composition should always be borne in mind, for we already know that in man the subcutaneous the genital, the mesenteric and the liver fat each have a characteristic composition. It has also been shown (Thannhauser and Boncoddo,1948) that brain sphingomyelin has a different fatty acid composition from that of other organs.

Johnson, McNab and Rossiter (1948b) examined peripheral nerve lipids with the micromethods used in their studies of

brain lipids. They pointed out that the concentrations of lipid fractions in peripheral nerves were less than in the central nervous system because of the presence of fibrous connective tissue in the peripheral nerves. All the lipids which they regarded as characteristic of the myelin of the central nervous system were present in the femoral and sciatic nerves in animals and man. These workers did not claim to be able to show the distribution of the lipids between axon and myelin, but an interesting observation was that in peripheral nerve, the sphingomyelin fraction of total phospholipid is approximately 50-70 per cent, compared with whole brain 20 per cent, and white matter 40 per cent. They concluded that on the whole the peripheral nerve lipids resembled those of white matter.

Johnson et al estimated lipids in normal peripheral nerve to provide standards for their subsequent work (1949) on the chemical changes taking place in Wallerian degeneration, in which destruction of the myelin sheath is a marked histological feature. The histological changes in this condition have been studied in great detail, but Johnson et al were the first modern workers to study the changes biochemically, as a means of understanding the chemical constitution of the myelin sheath. Their earlier work on the lipids of the central nervous system suggested that cholesterol, sphingomyelin and cerebrosides were the myelin lipids: their studies on degenerating peripheral nerves showed that these lipids decreased at approximately the

same rate and to the same extent, whereas cephalin decreased more rapidly and lecithin more slowly. Johnson et al presented these observations as further evidence that cholesterol, sphingomyelin and cerebrosides were the typical myelin lipids.

These results should be considered with those of Brante (1949) who also examined the lipids of nerves during Wallerian degeneration, and came to similar conclusions. Brante made some interesting observations in discussing his results, in which he compared the histological with the biochemical changes. Both he and Johnson et al found little lipid change at eight days, although at that time the axon had disappeared histologically, and did not conduct any impulses. Brante suggested that the axon lipids may have been absorbed into the sheath lipids and were then protected from autolysis in the early stages. The cellular proliferation which occurred during Wallerian degeneration must also be remembered when trying to deduce which structure released a particular lipid, and there could be no absolute value because of this variation in the histological picture.

The conclusion which may be drawn from these studies is that the central nervous system is rich in lipids, especially lecithin, cephalin, sphingomyelin, cerebrosides and cholesterol. Biochemical analysis shows that myelin lipids are probably sphingomyelin, cerebrosides and cholesterol. Studies on the lipids of peripheral nerves tend to confirm this. Chemical

analysis also shows that carbohydrates are important constituents of the cerebrosides of the myelin lipids.

We have seen that the lipid constituents of the myelin sheath have been known for a long time, and that modern methods of micro-analysis have made our understanding of them more complete. Similarly, the protein component of the sheath was recognised many years ago by Kühne and Chittended (1890) who described the neuro-keratin of the sheath. There has been much controversy over the histological appearance of this network in the myelin, and its presence has been ignored in many modern text books. It is best revealed by alcoholic fixation and haematoxylin staining. Cajal (1909) considered that the appearance was the result of histological manipulations, and many other neurohistologists were also of this opinion. Nageotte (1911) said that nothing is easier than the production of artificial networks in the myelin sheath. None of them denied, however, the presence of protein in the sheath; they were uncertain of its arrangement. There have been similar controversies about appearances in other tissues.

The problem was one which has been partly solved by biochemical and physical methods.

The early analyses of Kühne and Chittenden (1890) showed that medullated nerves contained a substance insoluble in alcohol and ether, and resistant to pepsin and trypsin. They estimated the amount in white matter as approximately 2.5 per cent (2.434-2.902) and in grey matter as 0.3 per cent of wet weight.

A long time passed before accurate chemical analysis were made which showed the presence of protein, as lipoprotein, in brain white matter. Folch (1951,1952) found that a lipoprotein remained in white matter after the extraction of lipids by chloroform and methanol. This lipoprotein was resistant to pepsin and trypsin digestion. Folch's analysis showed that the phosphorus of this lipoprotein was part of the brain diphosphoinositide, and he suggested that this substance corresponded to part of the neuro-keratin of Kühne and Chittenden. Logan, Mannell and Rossiter (1952) also suggested that diphosphoinositide was part of the myelin sheath. They studied the protein bound phosphorus compound of peripheral nerve during Wallerian degeneration, and found that in a crushed nerve the concentration of lipid P fell sharply 8-32 days after crushing. This was accompanied by disappearance of the inositide P. When regenerating nerves myelinated, the lipid P and inositide P reformed.

Finally the presence of extremely small amounts of copper in the central nervous system must be noticed (Eggleton, 1940). The distribution and function of this element within the central nervous system is not fully understood. It is possible that it is concerned with the maintenance of the myelin sheath.

HISTOCHEMICAL METHODS FOR MYELIN DEMONSTRATION

The histochemical methods considered for use in the present investigation are tabulated below and then examined in more detail, with an account of my personal experience with them.

Table 2 Histochemical methods for myelin demonstration

1 Choice of fixative.

2 Physical methods.

a Solubilities

1 Solubilities of lipids in organic solvents (Keilig, 1944).

2 Solubilities of lipids in coloured re-agents

Sudan black (Lison and Dagnelie, 1935: Leach, 1938).

Nile blue sulphate (Lorrain Smith, 1907: Cain, 1947, 1948: Menschik, 1953).

b Optical

Polarisation microscopy (Setterfield and Sutton, 1935: Lison, 1936).

3 Chemical methods.

a Unsaturated lipids

Osmium tetroxide (Hoerr, 1936: Bruesch and Arey, 1942: Cain 1950).

b Phospholipids and Cerebrosides

1 Metallic mordant/haematoxylin (Smith and Mair, 1909: Baker, 1946).

2 Periodic acid oxidation/Schiff reaction (McManus, 1946: Hotchkiss, 1946).

3 Metachromatic reactions (Feyrter, 1936: Wislocki and Singer, 1950).

c Cholesterol

Modifications of the Liebermann-Burchardt reaction (Schulz, 1924).

1. Choice of fixative.

Schmitt and Bear (1939) stated "It has long been obvious that the wealth of complicated structures demonstrated in the myelin sheath by various cytological methods, and the inevitable disagreement among cytologists as to which of these various pictures represents the structure of the sheath in the fresh state, are due to the extreme sensitivity of the sheath colloids to chemical and physical manipulations"(1939) Fixation, of course, is one of the manipulations which may disturb the structure of the myelin sheath. Unfortunately, if the sheath is to be studied in its relationship to other structures, fixation is essential for the production of good sections. All the methods tabulated above necessitate the use of histological sections, and are usually performed after the fixation of the material. One of the properties of a good fixing fluid is that it should preserve tissue components in their original configuration as nearly as possible, and because of the possibility of fixation artefacts, there has been much argument about the validity of interpretations in fixed preparations of tissue and cell morphology. Likewise, any chemical reactions which occur during fixation must be considered if interpretations of subsequent histochemical tests are to be valid.

Formalin is generally regarded as an excellent fixative for the central nervous system. It will be pertinent to consider the action of formalin on the myelin lipids, to learn

whether it alters the solubility of any particular lipid fraction, or if it has any direct action on any of them.

Weil (1929) demonstrated changes in the different lipid fractions during formalin fixation. He used human brains, and found that phosphorus appeared in the fixative within 24 hours in a proportion which corresponded to a measured decrease in brain phosphatides. After further investigation, he concluded that with formalin fixation of the central nervous system, the cholesterol and cerebrosides remained practically unchanged, but that phosphatides decreased considerably, due to hydrolysis. Brante (1949) reached similar conclusions. Kimmelstiel (1929) found a decrease in phospholipids extractable from the central nervous system of guinea pigs and rabbits after formalin fixation, with little change in cholesterol and cerebrosides. He explained the reduction as due to the lower "releasability" of phosphatides after fixation and not to hydrolysis, as he found insufficient phosphorus in the fixing fluid to account for the decrease in extractable phosphatide. This possibility that formalin might have reacted with phospholipids and then rendered them less extractable with lipid solvents was also suggested by Halliday (1939) in a study of the effects of formalin on liver lipids. Thus, there seemed to be no certainty of the chemical reactions involved during fixation of phospholipids with formalin.

Another effect of formalin on tissue lipids was that it

probably reacted at the double bonds of unsaturated lipids to produce free carbonyl groups. (Wolman and Greco, 1952). It is important to recognise this possibility when performing the Schiff test, or the procedure proposed by Ashbel and Seligman to visualise carbonyl groups (1949).

Of the other common fixatives, those containing alcohol or chloroform have obvious disadvantages in a study of the myelin lipids. The effects of osmium tetroxide on properties of peripheral nerve myelin have been studied by Finean (1954). He concluded that well-defined diffraction patterns were obtained from nerve fixed in buffered osmium tetroxide, but not from nerve treated with lipid solvents or formalin.

The conclusion can be drawn that although the chemistry of lipid fixation by formalin is imperfectly understood, it seems certain that lipid solubilities are altered by such fixation. My own histological experiments, described in a later chapter, confirm this.

Formalin is, however, the most useful fixative for whole brains, and is also used before several special procedures such as Weigert's myelin stain, Bielschowsky's silver impregnation method, and some of the modifications of the reduced silver method of Cajal. It is the fixative of choice in the present work.

2. Physical methods.

a. Solubilities

Some remarks are necessary on the solubilities of pure lipids, and on our knowledge of the physical state of tissue lipids. Folch and Sperry (1948) emphasised that the solubilities of different lipids were unsatisfactory criteria for differentiating them, even into groups. They pointed out that in using solubilities only as a definition, confusion might arise because lipid fractions from different sources might have widely differing compositions, and that even in a single source the solubility of one pure phosphatide might be influenced by the presence of another. An example is sphingomyelin, almost completely insoluble in diethyl ether, yet the presence of other phosphatides may make ethereal solution possible. Even when dealing with pure fatty acids, the most satisfactory solvent can only be specified if the particular fatty acid and the temperature of the reaction are known. The solubilities cannot be predicted when two or more fatty acids are present. The water solubility of a fatty acid is, in general, dependent on the length of its hydrocarbon chain. The acids with more than eight carbon atoms are only very slightly soluble in water but the carboxyl group even in long chain fatty acids tends to oppose the action of the hydrocarbon chain.

The significance to histochemistry of differences in behaviour between a pure substance and the same substance in a mixture is evident.

Equally important, if histochemical methods are used, is some knowledge of the physical state of the substances investigated. Unfortunately, the physical state of tissue lipids is not fully understood. Serum lipids have been extensively investigated, for the good reason that they are easily accessible, but detailed knowledge of other tissue lipids is incomplete. It will be shown that the fully developed myelin sheath can be demonstrated by several histological methods; in the same way fat granules in leucocytes and fat droplets in sebaceous glands are demonstrable. Myelin, fat granules, and droplets are some of the so-called "visible" tissue lipids. On the other hand, similar methods may fail to demonstrate lipids in some structures in which biochemical analysis shows that considerable amounts are present. Such organs are kidney, liver, and heart muscle, and their fat is often referred to as "masked" lipid. Histological methods fail to show these lipids, perhaps because they are combined as lipoprotein, or perhaps are extremely finely dispersed. The physical state of the fully developed myelin sheath has been investigated, and shown to consist of alternate layers of lipid and protein. (Schmitt, Bear, and Palmer, 1941). The important questions to ask in the present investigation, when any histochemical method is proposed, are whether the reagents used can reach and penetrate the lipids, and, if such penetration is possible, is the state of dispersal of the lipids such that they are visible with the ordinary light microscope. It

is possible that many reagents may be held up at the protein layers and not penetrate to the lipids. (J.B.Finean, personal communication).

Keilig (1944) made use of lipid solubilities (based on Table 3) for her work on the specificity and basis of methods for myelin staining.

Table 3. Lipid solubilities

	Acetone Cold	Acetone Hot	Ether Hot	Chloroform/Absolute alcohol Hot
Glycerol esters	+	+	+	+
Cholesterol esters	+	+	+	+
Phosphatides				
Lecithin	-	-	+	+
Cephalin	-	-	+	+
Sphingo myelin	-	(+)	-	+
Cerebrosides	-	+	-	+

Keilig seems to have been the only worker to have used the method of fractional extraction to decide what myelin constituents are responsible for producing the colour reaction in Weigert's myelin stain and its modifications. Bielschowsky (1935) stated that lecithin was responsible for the colouration, but Keilig concluded that the cholesterol of myelin played an essential part in these staining procedures. She also found

that the Weigert staining reaction was completely negative after chloroform/alcohol extraction, except for the neurokeratin of the sheath. If it could be generally agreed that Table 3 was a reliable one, her results would be valuable,but we know that the solubilities of lipids in mixtures are not accurately predictable and I decided against the use of any fractional solution methods for this reason. Keilig's methods were an over-simplification of what is a difficult problem, the qualitative examination of tissue lipids. Yet if biochemists and histologists worked together on such problems as the action of formalin on the myelin lipids the unsatisfactory state of our knowledge of myelin staining methods might be resolved. Hurst (1953) stated that "the current stains for myelin are sadly lacking in their ability to reveal the finer differences in a morbid anatomical picture". This is partly because we are not even sure what part of the myelin still remains after various histological procedures, and so cannot be certain what myelin constituents are responsible for the colour reactions of the various staining methods.

There is, then, biochemical and histological evidence to show that the solubilities of lipids are not satisfactory criteria for exact histochemical tests. However, the solubility in lipids of certain colorants (sudan IV; sudan black; nile blue sulphate;) is a physical reaction which makes a valuable histochemical method for demonstrating myelin lipids.

Sudan black, a powder of the azo dye group, was

introduced by Lison and Dagnelie (1935). It is soluble in 70% alcohol, and passes out of a saturated alcoholic solution to dissolve in lipids, colouring them blue-black, and rendering them visible in histological sections. Lipids only are coloured, all other substances remaining uncoloured. The action is reversible, and the sudan black can be removed from the section by immersion in 70% alcohol, or very rapidly by higher grade alcohols or acetone.

There are technical, and theoretical objections to its use, as there are to all dyes of the sudan group. One technical objection is that there is a tendency for the production of precipitation artefacts. Cain (1947) used sudan black for a study of the phospholipids of the Golgi apparatus. As controls he stained sections of cigarette paper, and obtained an exceedingly faint colouration, which may have corresponded to fine precipitates, or absorbed lipids in the paper. I find that with the use of a freshly prepared filtered solution, and staining in a closed receptacle for 10 minutes, followed by a rapid wash in 70% alcohol, perfectly clean sections are obtained. A second technical objection is to the use of sudan black in a solution which itself may have a dissolving effect on fine lipid droplets in the sections. Kaufmann and Lehmann (1926) used sudan III in a 40% alcoholic solution in an extensive study of specific methods for fat staining, and obtained positive staining results with a large range of pure lipids. They thought the use of 70% alcohol might extract

fine lipid droplets. For the same reason Leach (1938) recommended the use of excess sudan black in a 50% solution of diacetin. He found such a solution stained myelin intensely. Telford Goven (1944) used colloidal solutions of sudan III for lipid staining. Substituting sudan black for sudan III in colloidal solution, I find no advantage in such solutions for demonstrating fine myelin sheaths.

Are there any objections to the claim of sudan black as a specific method for demonstrating tissue lipids? Its property of colouring lipids is, as mentioned above, due to a physical reaction, for the coefficient of partition between 70% alcohol and lipids is greatly towards the latter. No other substances are coloured by it, but attempts to translate this physical property into an explanation of the reactions of tissue lipids are not simple. There may be unknown non-lipid substances present in tissues, having a similar physical property; and experiments with pure preparations of the lipids known to be present are not always valid. Cain (1950) criticised the experiments on pure lipids and lipid mixtures of Kaufmann and Lehmann (op.cit.). He considered that the "pure" substances investigated probably contained impurities; that some substances being investigated were lost during handling; that the physical state of some of the mixtures was such that the staining reagent under test could not penetrate; and, most important, that the properties of mixtures of lipids were not deducible from those of their components.

Kaufmann and Lehmann investigated, among other reagents, sudan III and not the more sensitive sudan black, with which there have been no comparable experiments on pure substances and mixtures.

The possibility of false positives must be considered. Berenbaum in a preliminary report (1954) of work on lipo-protein complexes enumerated some very diverse substances as blackening with sudan black after treatment of up to 12 hours. These included collagen and reticulin. While it is probable that reticulin contains lipid (Little and Windrum, 1954) there is no chemical evidence that collagen does. I find that the septa in the anterior part of the optic nerve never take up the sudan black. They stain an intense red with van Gieson's stain.

A final objection to the use of sudan black in the study of the myelin lipids is that it cannot react with lipids that are solid at room temperature. The melting point of pure cholesterol is 148.5°c.: sphingomyelin, 196°c.: and the cerebrosides also have high melting points. Thus, in theory, the most important constituents of the myelin sheath cannot stain with sudan black. Therefore either some other myelin constituent is staining or the physical state of the myelin lipids is so altered by the presence of other substances, e.g. lipo protein complexes, that their melting points are different from those of the pure substance. This latter is quite possible (Dr. G. Boyd, personal communication).

Before introducing sudan black as a new method for

colouring myelin, Lison compared many sections stained by his method with controls stained by Weigert's method. He found that sudan black stained the finest myelinated fibres, and he made particular comparison between the methods in their staining of the "couche supraradiaire". Weigert himself had found the myelinated fibres difficult to demonstrate in this region where Lison said sudan black showed the myelin well.

Brodal and Harrison (1948) used sudan black staining as one method of studying the chemical composition of myelin, and the smallest size of myelinated nerve fibres in the central nervous system, but they found sudan black less sensitive than acid haematein as a myelin stain. Sudan black was also used by Noback and Montagna (1952) for histochemical studies of the myelin sheath during Wallerian degeneration.

I find that sudan black demonstrates extremely fine myelinated fibres, down to 1μ in diameter, and because of the simplicity of the method and its specificity for lipids, it is the method used for the demonstration of myelin in the present investigation.

Another dye which has had wide use as a lipid colorant, is nile blue sulphate. Lorrain Smith (1907) first introduced it in a method to distinguish neutral fats, which stained red, and fatty acids, which stained blue. The dye consists of a blue oxazine which is oxidised to a red oxazone by boiling with dilute sulphuric acid. The specificity originally claimed for it has been challenged by Kaufmann and Lehmann(1926)

and Lison (1936). Cain (1947, 1948) however, claimed that the blue colouration is specific for acidic lipids i.e. free fatty acids and phospholipids. Nile blue sulphate was used as a myelin stain by Brodal and Harrison and Noback and Montagna in their studies referred to above. Menschik (1953) also introduced a nile blue histochemical method for phospholipids. My objection to this method was that it depended on the premise that all lipids other than phospholipids were soluble in acetone after fixation.

b. Optical

Polarisation microscopy as a method of identifying lipids is of doubtful value. Gomori (1952) stated that the underlying causes of birefringence in mounted histological sections were not fully understood, and different factors, such as the state of lipid aggregation and the nature of the mounting medium, must be considered.

Lison (1936) also decided that birefringence must be interpreted with caution, and that the results were of little value in distinguishing neutral fats, fatty acids, or phospholipids. Cholesterol also had varying behaviour depending on its physical state.

Setterfield and Sutton (1935) and Noback and Montagna (1952) examined the birefringence of peripheral nerve during Wallerian degeneration. Noback and Montagna used other methods also, but I found that it was difficult to correlate the appearances they found with staining methods, with those obtained from polarisation microscopy.

3. Chemical methods.

One of the oldest methods of demonstrating the myelin lipids is by the use of osmium tetroxide. This is a strong oxidising agent, almost colourless in weak aqueous solution, but becoming black when reduced. It is used to demonstrate lipids because the presence of numerous double bonds in unsaturated fatty acids makes them easily oxidisable, with consequent reduction and blackening of the osmium tetroxide. The technique, briefly, is to fix the tissue in osmium tetroxide, dehydrate, clear, and embed. Sections are cut and after removal of the paraffin are ready for mounting. Bruesch and Arey (1942) recommended the use of the vapour of a 2% solution of osmium tetroxide as a fixing and staining reagent for myelin sheaths, and were able to count the fibres in the optic nerve after this method of staining. Osmium tetroxide in buffered solution has been used as a fixing agent by Palade (1952), and Hess and Lansing (1953) also used osmium tetroxide for electron microscope studies of the fine structure of peripheral nerve fibres. There is no doubt that it can give a beautiful morphological picture, and Finean (1954) was of the opinion that structures revealed by electron microscope studies of peripheral nerve fixed with osmium resembled closely the original structure.

Other workers have objected to the use of osmium as a specific method for demonstrating lipids (Hoerr,1936: Lison 1936). One weakness in its use as a specific agent is that

some lipids, previously negative, give a positive blackening only after treatment with alcohol. An example is lecithin. Another difficulty is that all tissue elements cause blackening if the reaction proceeds for a long time. Finally, it is difficult to explain the action of chromates with osmium tetroxide. If the osmic solution contains potassium dichromate, or if chromates are used before the osmium, normal myelin remains unblackened, but degenerating myelin will blacken. This, the method of Marchi, is extremely useful in pathological work, but there is no chemical basis for it, (Johnson, McNab and Rossiter, 1950) and it cannot be used to "label" any of the products of the degenerative process.

My personal experience of the method is limited, for I find the penetrating power of osmium tetroxide as a fixative is too low. I can only obtain a satisfactory histological preparation with small pieces of tissue, with the method of Bruesch and Arey (1942).

Methods claiming to be specific for phospholipids are based on the principle that chromates in the fixing fluid immobilise and mordant phospholipids, producing an extremely insoluble substance which is then able to form a visible blue-black compound with haematoxylin. Differentiation with borax ferricyanide is necessary. (Smith and Mair, 1909: Baker, 1946). There are two objections to the principles of these methods. The first applies to any "indirect" method depending on the formation of a visible intermediate product. There is always

the possibility that the intermediate product may diffuse out, or become absorbed at some locality other than the site of origin. Baker's extensive studies (1946) on the solubility of phospholipids after using the formaldehyde/calcium mixture and chromation in his technique indicated that there was no diffusion of intermediate products from the section. He also evolved a control technique to distinguish certain proteins which also colour blue-black and tend to interfere with the results. I cannot see that Baker's control method was perfectly valid, for he used a different fixative for the "control" and the test. A second objection is to the need for differentiation. This is a perfectly legitimate process when fine detail is not being studied, but it seems dangerous when the earliest signs of a specific substance are sought. After preliminary trials with Baker's acid haematein method, I did not continue with it for that reason. I have therefore not used any metallic mordant/haematoxylin method in this study, although I find that a simple Weigert haematoxylin stain on frozen sections demonstrates fully developed myelin sheaths perfectly well, without any mordanting or differentiation.

Another indirect method which demonstrates phospholipids and cerebrosides depends on the conversion of adjacent hydroxyl groups, if present in any molecule, to an aldehyde group after oxidation. The aldehyde groups are then rendered visible as a purple compound by further reaction with Schiff's reagent. The methods introduced by McManus (1946) and Hotchkiss (1946)

used periodic acid as the oxidising agent (PAS reaction). This reaction was originally used to demonstrate acid and mucopolysaccharides. The molecular structure of the nitrogenous base sphingosine, and the presence of carbohydrate in cerebrosides are theoretical reasons why certain lipids should also give positive results. Jeanloz (1950) considered the chemical explanation of the PAS reaction as unsatisfactory. He found that not all polysaccharides with adjacent hydroxyl groups gave a positive reaction, and the strength of positive reactions did not depend on the amount of periodic acid consumed by the theoretically available adjacent free hydroxyl groups. However, Wolman (1950) has shown that unsaturated lipids and sphingolipids stain with Schiff's reagent after periodic acid oxidation, both with experiments on pure compounds and histological material.

Hess (1953) stated that white matter was definitely PAS negative, but he used pieces of central nervous system fixed in Rossmann's fluid (absolute alcohol saturated with picric acid 90 cc.: formaldehyde 40%, 10 cc.). This again illustrates the importance of accurate knowledge of the fixation reactions when studying lipids histologically. My own observations show that the method demonstrates myelin well in frozen sections after formalin fixation, and I used it extensively in the present investigation, always remembering that it is an "indirect" method.

One other method for phospholipids and cerebrosides remains to be considered, the metachromatic staining reaction. Metachromasia may be defined as the staining by a pure dye of some components of a tissue section in a colour markedly different from that of the remainder of the section, and from the colour of the original dye itself. Examples of such dyes are thionin, toluidine blue, and methylene blue; and cartilage and epithelial mucin are examples of substances which stain metachromatically. A considerable amount of work has been done to elucidate the theory of metachromatic staining. It is not due to impurities in the dye, or to a mixture of substances. Michaelis and Granick (1945) stated that the metachromatic dyes existed in different states of polymerisation, and that the different polymers had different colours. Polymerisation of the substrate caused polymerisation of the dye. Lison (1936) thought that the reaction was specific for sulphuric acid esters of high molecular weight (acid mucopolysaccarides). Whatever the theoretical basis of the reaction may be, it is necessary to consider in detail the techniques used by various workers. This is particularly important because in this method slight technical variations cause very differing results.

Feyrter (1936) and Wislocki and Singer (1950) have used the metachromatic staining method to demonstrate the myelin sheath of peripheral nerves. Their method will be outlined before making some criticisms of conclusions drawn from its use.

Feyrter cut frozen sections from formalin fixed material, stained and mounted them in a watery thionin/tartaric acid solution, and surrounded the cover slip with paraffin to prevent evaporation of the staining medium. Myelin sheaths stained pink, in contrast with the original blue due. Feyrter claimed that the metachromasia was due to the presence of cerebrosides. Wislocki and Singer (1950) substituted toluidine blue for thionin, and associated the metachromasia with the presence of sulphatide groups in the myelin sheath.

The step in the technique of the metachromatic reaction about which there is the greatest difference of opinion is whether sections should be dehydrated after staining with the metachromatic dye. Feyrter, and Wislocki and Singer mounted their sections without dehydration. Sylven (1941) used the method in work on granulation tissue and epithelial regeneration, and declared that "true" metachromasia was only evident after alcoholic dehydration, and that metachromasia shown in watery mounting was not specific. Penney and Balfour (1949) who worked on wound healing, also found that metachromasia disappeared after alcoholic dehydration and they only con - sidered the metachromasia still present in sections after such dehydration. Holmgren and Rexed (1946) studied the meta- chromasia of Schwann cells in nerve regeneration. They used alcoholic dehydration, and with their methods the myelin sheath failed to stain metachromatically, although the cytoplasm of the proliferating Schwann cells did so.

Criticism of Feyrter was raised by Pischinger (1943). After extensive experiments he declared that formalin in the sections, remaining after fixation, was responsible for the metachromasia which Feyrter claimed was due to cerebrosides of phospholipids. In this connection, it is interesting to note a combined fixation/staining method introduced by Chang (1936). He used a fixation/staining solution of thionin in formaldehyde, and said that after normal dehydration, clearing, and embedding, sections showed that cells stained blue and tracts red. He claimed no histochemical qualities for the method.

Using Feyrter's method on formalin fixed, frozen sections of optic nerve, I find that the pink colour of the myelin sheaths is lost immediately on alcoholic dehydration. This is not because, as Wislocki and Singer (1950) said, the myelin lipids are soluble and disappear. I find that the pink colouration reappears if the section is hydrated and again placed in the staining solution. The preparations are not permanent; fine diameter fibres are extremely difficult to examine; and the specificity of the method for cerebrosides and phospholipids seems uncertain. I therefore do not use the method extensively. The only excitement I had with this method was with optic nerves of a 20 week foetus. After staining with toluidine blue followed by dehydration some beautiful metachromasia was seen. At first I thought this was a sign of early myelin formation in association with

proliferating cells, but my final conclusion was that the metachromasia was associated with proliferating capillaries.

The method warrants further detailed investigation both in its application to the histochemistry of the normal nervous system, and to clarify the histological appearances in the group of diseases known as the metachromatic leuco encephalopathies. However, Lumsden (1951) jumped ahead of the available evidence when he said "The metachromatic substances present in the leuco dystrophies are, or are related to, galacto sphingosides or cerebrosides, and the oligodendrocyte is intimately concerned with the metabolism of these substances."

For the purpose of this investigation, a reliable histochemical method for cholesterol would have been invaluable, but I can not find one suitable for demonstrating small amounts of this myelin lipid. With the Schulz modification of the Liebermann Burchardt test for cholesterol, there is a preliminary mordanting of frozen sections in 2% ferric alum for 24 hours. A mounted section is thoroughly dried and then covered with a drop of concentrated sulphuric acid, followed after 3 - 15 seconds by 2 - 3 drops of acetic anhydride. A positive result is indicated by the appearance of a colour changing through blue to green.

With sections from adult central nervous system, I find the results quite unpredictable, and charring of a large proportion of the sections is another difficulty. I find the method unsuitable for the demonstration of fine lipid changes in tissues.

MATERIAL AND METHODS

Human and animal material was examined.

Table 4 shows the ages and regions of the human foetal material examined. I confined the selection of material almost exclusively to optic nerve and chiasma, and medulla, because these regions were most easily identified, and subjected to least damage during post-mortem examination. Such examination necessarily included careful inspection of the falx cerebri, tentorium cerebelli, and venous sinuses. After this, it was not always possible to be accurate about the topography of the cerebral hemispheres. Preliminary examinations also showed that these areas myelinated at a suitable time for the purpose of the present investigation.

Brain stem and cerebellum of 9 sheep foetuses were examined. The crown-rump length of these foetuses ranged from 13 cm. to 31 cm.

The previous chapter reviewed and criticised the histochemical methods available for the demonstration of the myelin sheath and its constituents, and explained my selection from such methods.

In addition, the following methods were also used:
Haematoxylin and eosin.
Van Gieson for connective tissue.
Phosphotungstic acid haematoxylin for fibrous processes.
Silver impregnation (Gordon Sweet) for reticulin.
Silver impregnation(Hortega silver carbonate)for oligodendroglia.
Gold sublimate (Cajal) for astrocytes.

TABLE 4

Human material examined.

No material was included in this series if more than 24 hours had elapsed post-mortem, if there were clinical or pathological signs of intra-uterine death, or if the cause of death was known to have interfered with lipid metabolism.

Foetal age weeks		Parts examined
20		Optic nerve
23	1	Medulla oblongata
	2	Medulla
		Optic chiasma
24	(survived 11 hours)	Optic nerve
		Oculomotor nerve - (peripheral nervous system)
26	(survived 7 weeks)	Optic nerve
30	1	Medulla
		Optic chiasma and nerve
	2	Medulla
		Optic chiasma and nerve
		Occipital cortex
31	1	Medulla
		Optic chiasma
		Sciatic nerve
	2	Optic chiasma and nerve
32	1	Optic chiasma and nerve
	2	" " " "
36	1	Medulla
		Corpus callosum
		Optic chiasma and nerve
	2	Medulla
38	1	Medulla
	2	"
	3	"
Term	1	Medulla
		Corpus callosum
	2	Optic chiasma and nerve
	3	Optic chiasma and nerve
+36 hours		Optic nerve
+ 3 weeks		Medulla
+ 4 months		Optic nerve
+ 5 months		Medulla

RESULTS

Results are first presented of some preliminary histological experiments undertaken to examine the effects of organic solvents on the lipid staining properties of myelin sheaths, before and after formalin fixation.

Material Brain stem, cortex, and spinal cord of human adults and foetuses.

Group 1 Blocks approximately 1 cm. thick were fixed in 10% formalin and then -

(i) Washed in running water.

(ii) Frozen sections cut at 15 u.

(iii) Stained in a saturated solution of sudan black in 70% alcohol for 10 minutes.

(iv) Rinsed in 70% alcohol for 15 seconds.

(v) Washed in distilled water.

(vi) Mounted in a water mounting medium.

Group 2 Slices approximately 5mm. thick were washed in water, then divided into two further groups.

2a Immersed in a large volume of a 2/1 chloroform absolute alcohol mixture for 24 hours at room temperature in a shaking machine.

2b Dehydrated through ascending grades of alcohol and then fixed in acetone for 24 hours.

(i) 2a Washed in running water for 8 hours.
2b Taken down to water.

(ii) Immersed in 10% formalin for 24 hours, and subsequently as in Group 1.

Group 3 Frozen sections were obtained according to the routine in Group 1 but before staining with sudan black they were passed through ascending grades of alcohol, and approximately six were placed in each of the solvents: - absolute ethyl alcohol: xylol: alcohol/chloroform: acetone: ether: and pyridine, for 24 hours at room temperature. After returning down to 70% alcohol, sections were stained for lipids with sudan black, as in Group 1.

Sections from Group 1 show the histological picture obtained by the routine use of sudan black on formalin fixed frozen sections. (Figs. 1, 5, 7).

Sections from Group 2 (a) show that no recognisable myelin sheaths remain after extraction with a chloroform/ alcohol mixture before the action of formalin. Sudanophilic material is still present. (Fig. 2).

Sections from Group 2 (b) show an unsatisfactory histological picture. Although myelin sheaths are still recognisable, the background is blurred and dirty. (Fig. 3).

Sections from Group 3 show that myelin sheaths are still recognisable after formalin fixed material is treated with lipid solvents. (Figs. 4, 6, 8).

I therefore conclude that some of the myelin lipids remain in formalin fixed material which is treated with fat solvents. What remains after extraction with fat solvents can only be accurately determined by biochemical analyses. Deductions based on staining methods are unsatisfactory.

I also conclude that even if a group of lipids can be selectively removed from unfixed material (and the biochemical evidence is against this premise), the remaining lipids may diffuse from their original sites, and the histological picture becomes so distorted that accurate cytological localisation is impossible.

The remaining results are presented as a purely descriptive account of sections stained by histochemical and other methods to demonstrate changes in certain parts of the central nervous system during the deposition of myelin around axons. Characteristic lipid changes are described in detail only at certain stages in development, for the variation in the appearances of the lipid changes is quantitative. This variation is correlated with the variation in number of the myelinating fibres, and presented in tabular form.

The interpretation of these appearances, and the validity of regarding them as directly related to myelination is discussed in the following chapter.

Human material.

20 weeks foetus

Optic nerve, anterior third only.

Sudan black. No myelin tubes are present in this part of the nerve. A delicate sudanophilic network is seen in a transverse section, but no lipid droplets or larger aggregates are evident.

Van Gieson. This method also demonstrates the presence of a delicate fibrous network in the nerve, and in addition, shows its relationship to the numerous cells. The processes of the network do not stain like collagen and are apparently not always connected with cell bodies. Coarser septa carrying blood vessels into the nerve divide it into segments, within which the finer network is still visible. These septa were unstained in the sudan black preparations. The septal cells are typical fibroblasts, and are unrelated to the cells of the fine network. (Fig. 9: see also Fig. 10).

Azur A. A strong metachromatic reaction is given in those septa in which capillaries are visible, but nowhere else.

23 weeks foetus

Optic chiasma.

Sudan black. The sudanophilic network is present. Minute lipid droplets are seen on the strands of the

network. At this stage, the droplets cannot be shown to have any relationship to cells in their vicinity. Isolated myelinated fibres can be seen. (Fig. 11).

Developing myelinating fibres have a characteristic nodular appearance. (Figs. 30 - 33). The earliest myelin sheath nodules are arranged in beaded columns. The interval between the nodules increases, and the nodules finally disappear.

30 weeks foetus

Optic chiasma.

Sudan black. Lipid droplets along the network are more numerous than in the 23 weeks foetus. Larger lipid droplets have made their appearance. (Figs. 15 - 17). Some of these deposits are seen to be closely associated with a myelinating fibres. When this is so, it is not always possible to decide whether the myelin of a particular axon is formed completely independent of, and separate from the myelin of a neighbouring axon.

The lipid deposits do not give a positive periodic acid Schiff reaction.

Some of the deposits, but not all, are doubly refractile with polarised light.

Methylene blue. Cells are seen to be arranged in the chiasma in columns. Cells forming a column are closely packed, and the columns themselves are also

arranged extremely closely. The cells are almost spherical, with a large nucleus.

Medulla.

Sudan black. Similar lipid depositions to those seen in the chiasma are seen in the medulla. They are confined to the pyramidal region. (Fig. 14) Myelin sheaths are also present in the pyramid.

The relationship of these sudanophilic deposits to cell nuclei is demonstrated in sections stained with sudan black and counterstained with alum haematoyxlin. Many of the deposits are intracellular. (Fig. 18).

38 weeks foetus

Medulla

Sudan black. The large sudanophilic deposits in the pyramidal region are still present, but they have decreased in number compared with the 30 weeks foetus. The myelinated fibres in the pyramid have increased greatly in number.

Phosphotungstic acid haematoxylin. The fine network is demonstrated by this method. (Fig. 13).

Reticulin. The network is not true reticulin, for although silver can be deposited on it, the picture obtained is not comparable with the reticulin of the blood vessels in the same area. (Fig. 12).

Hortega silver carbonate. Chains of nuclei are demonstrated between the pyramidal bundles, but cell processes are difficult to impregnate. (Fig. 28).

Cajal gold sublimate. Very few astrocytes are seen between the pyramidal bundles. They are present in adjacent areas. (Fig. 29).

Foetus at term

Optic chiasma.

Sudan black. Myelination is heavy and lipid concentrations are no longer visible. (Fig. 19).

The appearances in optic chiasma detailed above are described from horizontal sections, and those of the medulla from sagittal sections. This method of cutting these regions was selected in order to be able to examine different levels of a tract in one section, for it is possible that visible lipid changes associated with myelination, if any, might vary in different localities of the same tract at one time.

We see that myelinated fibres are present in the chiasma of the 23 weeks foetus. Table 5 shows the variation during development in the quantity of the myelinated fibres in the chiasma and nerve. An exact numerical evaluation is not attempted, but the nerve is estimated as showing from a few myelinated fibres (trace) to heavy myelination (++). A

similar method of evaluating the quantity of the larger lipid deposits is also adopted: the maximum number of lipid deposits seen in any section of any age is designated ++, and the other estimations are based on this.

This method of correlating lipid deposits and myelinated fibres by an estimation expressed in inexact terms has the disadvantage of being purely subjective. The myelinated fibres could be estimated numerically, but it would prove very difficult to count the lipid droplets and deposits.

TABLE 5

Myelinated fibres in human optic nerve and chiasma.

Foetal age.	Survival	Myelinated fibres chiasmal end	Middle	Globe end
23 weeks	Still-born	trace	- not available -	
24 "	11 hours	trace	trace	trace
26 "	47 days	trace	trace	trace
30 " 1.	Still-born	trace	trace	0
2.	"	+	trace	0
31 " 1.	"	+	- not available -	
2.	"	+	trace	0
32 " 1.	"	+	+	0
2.	"	+	+	0
36 "	"	++	+	0

further subdivision of middle

Term +	24 hours	++	++		+	trace
+	24 hours	++	++	++	+	trace
+	36 hours	++	++	++	+	+

All sections were frozen and stained with sudan black. When the nerve was divided, sections were cut and received into separate dishes. For the material at term, I did in fact make further subdivisions than are enumerated above, but I did not think I was able to estimate the differences in such subdivisions.

TABLE 6

Human material

Variation in quantity of lipid deposits and myelinated fibres.

A Medulla; pyramid

Foetal age	Lipid deposits	Myelinated fibres.
23 weeks 1.	0 (Fine droplets only)	trace
2.	0 (Fine droplets only)	trace
30 weeks 1.	++	trace
2.	++	trace
31 "	++	trace
36 " 1.	+	++
2.	+	++
38 " 1.	trace	++
2.	trace	++
3.	trace	++
Term	0	++
" + 3 weeks	0	++
" + 1 month	0	++
" + 5 "	0	++

B Optic chiasma

Foetal age	Lipid deposits	Myelinated fibres
23 weeks	0 (Fine droplets only)	trace
24 weeks (chiasmal end of nerve)	0 (Fine droplets only)	trace
26 weeks (chiasmal end of nerve)	0 (Fine droplets only)	trace
30 weeks 1.	++	trace
2.	++	+
31 " 1.	++	+
2.	++	+
32 " 1.	++	+
2. (chiasmal end of nerve)	++	+
36 weeks	+	++
Term + 24 hours	0	++
" + 24 hours	0	++
" + 36 hours	0	++

Animal material

Nine sheep foetuses which had been formalin-fixed were examined.

The regions selected were the lower border of the pons, in transverse section, and cerebellum in sagittal section near the mid-line. In the pons, the courses of the abducent, facial, and vestibular nerves could be recognised, and the cerebellar folia supplied compact areas of white and grey matter. It was thought necessary to study the possible occurrence of lipid-containing cells in grey matter. If lipid cells were present in large numbers in potential grey matter, their specific function in the formation of myelin was doubtful.

Before tabulating the results to compare the quantity of myelinated fibres and lipid cells, some aspects of typical sections will be described.

22cm. foetus

Transverse section, lower border of pons.

Sudan black. Numerous fibres are already medullated in the root of the seventh nerve beneath the facial colliculus, and cells containing lipid are seen along the course of the medullating fibres. Many fibres show the beaded appearance associated with early myelination. (Fig. 23).

24cm. foetus

Sagittal section through medulla.

Sudan black. Similar cells containing lipid are seen related to myelinating nerve fibres. (Figs. 20 - 22).

Cerebellum.

Sudan black. The white matter of the folia contains numerous medullated fibres. Lipid cells are also present. They are seen also in the granular and molecular layers. (Fig. 25).

27cm. foetus

Transverse section through lower border of pons.

Sudan black. The whole course of the seventh nerve in the pons is now well medullated. Lipid cells have decreased greatly in number. (Fig. 24).

TABLE 7

Animal material

A Transverse section of lower part of pons.

Crown-rump length		Medial longitudinal bundle		Pyramidal bundles		Nerve 6 abducens		Nerve 7 facial		Nerve 8 vestibular	
13 cm.		No myelinated fibres.				No cells containing lipid.					
20 cm.		+	++	+	++	+	++	+	++	+	++
22 cm.	1	++	+	+	++	++	+	++	+	++	+
	2	++	+	+	++	++	+	++	+	++	+
25 cm.		++	+	+	++	++	+	++	+	++	+
26 cm.	1	++	trace	++	+	++	+	++	trace	++	trace
	2	++	"	++	+	++	+	++	"	++	"
27 cm.		++	"	++	trace	++	trace	++	"	++	"
31 cm.		++	"	++	"	++	"	++	"	++	0

++ = myelinated fibres

++ = lipid cells

B Cerebellum

Crown-rump length		Nerve fibre layer		Granular layer		Molecular layer	
20 cm.		trace	+	0	0	0	0
22 cm.	1	"	+	0	0	0	0
	2	"	+	0	0	0	0
25 cm.		+	+	0	+	0	+
26 cm.	1	++	+	0	+	0	+
	2	++	+	trace	+	0	+
27 cm.		++	+	"	+	0	+
31 cm.		++	trace	"	trace	0	trace

++ = myelinated fibres

++ = lipid cells

DISCUSSION

The histological appearances described in the previous chapter must now be interpreted, and their significance assessed. Where possible, indication will be made of the need for further investigation of some problems arising out of this study.

An observation made by Hess and Lansing (1953) must be borne in mind when interpreting these sections made from fixed material. They said "The removal of a nerve from the body and treatment with a fixative is likely to produce drastic changes in a system as delicately balanced as a nerve fibre. At the moment, these are limitations that must be accepted." They were referring to peripheral nerve, but the remark is equally applicable to central nervous system.

Three main features of the sections must be considered:

1. The structural nature of the background in which the myelinating fibres lie.
2. The lipid changes in this background.
3. The morphology of the developing myelin sheath.

1 The structural nature of the background.

My explanation of the reticular appearance of the background, demonstrated by various staining methods, is that the network is made up by glial cell processes. (Figs. 9, 10) Examination with the oil immersion lens of counterstained sudan black preparations, and focussing at different levels in one field of view, leads me to believe that axons may actually pass through individual glial cells, that is, between the cell nucleus and cell wall. I conclude that there exists around unmyelinated axons a framework on which lipids can be deposited before investing the axons. The framework gives a positive reaction with sudan black and is also demonstrated by phosphotungstic acid haematoxylin. The framework is therefore probably lipoprotein in nature.

Sections of optic chiasma of a 30-36 weeks human foetus, stained with a nuclear stain such as methylene blue, show columns of cells lying in almost direct contiguity with each other. The cells are almost spherical, with a large nucleus. Methylene blue demonstrates cell bodies, but I can not demonstrate all cells and processes in one preparation. Hortega silver carbonate preparations for oligodendroglia are unsatisfactory in this respect, for they do not show processes corresponding to those shown in van Gieson or phosphotungstic acid haematoxylin preparations. In contrary manner, the latter type of preparations, although demonstrating a dense network, does not apparently stain all the nuclei. On fine

focussing with a high power lens, unstained cell outlines can be made out.

It is pertinent to compare the morphology of the cells I describe with the description by Lumsden and Pomerat (1951) of normal oligodendroglia in tissue culture. Using explants from rat corpus callosum, they found that oligodendroglia were polyhedral, pyramidal, or nearly spherical in shape, with a comparatively large spherical nucleus. Each cell had from three to five processes which, in length, were almost twice the diameter of the cell. Lumsden and Pomerat also suggested that the terminals of oligodendroglia processes were the precursors of processes that surround the myelinated fibre. Hogue (1947,1953) described oligodendroglia cells in similar terms.

Breemen (1954) in electron microscope studies of neuroglia cells also suggested that axons may pass through glial cells.

There is also evidence provided by other work. Alexander (1938) studied the neurone by micro-incineration methods. In a human optic tract, he found an outstanding mineral content, corresponding to glial cell nuclei. He also found that mineral-containing dots passed from glia to myelin sheath, and concluded that glial processes reinforced the sheath. Davis (1940) also concluded that in the optic nerve the oligodendroglia processes formed intimate contact with individual myelinated fibres. He studied normal histology as a

preliminary to a study of glial tumours.

My description of a glial network is not a revival of the old controversy about the presence or absence of a glial syncytium in the central nervous system. Warren Andrew (1949) reviewed this problem, and after further work considered that there was strong evidence of close physical contact, if not complete fusion, of the ends of the finest glial fibres.

It thus seems reasonable to regard the network as being of glial nature, if my histological results are considered with the conclusions of the workers mentioned above.

One other interpretation of the nature of the network should also be considered. It is possible that there is delicate protein layer, the axolemma, between the adult axon and the myelin sheath. Nageotte (1910) claimed to have demonstrated such a layer histologically. Hess and Lansing (1953) after an examination of peripheral nerve with the electron microscope, decided that a well defined membrane was present between the axon and myelin sheath. It is therefore possible that the appearances in transverse sections of optic nerve, for example, are due to disorganisation of the outer layer of the axon during fixation.

I prefer the glial network explanation, because it agrees with observations made on the structure of the living cell. The presence of a distinct membrane between axon and myelin sheath has still to be proved beyond reasonable doubt.

I must explain why I think it necessary to examine

sections for reticulin. Murray and Stout (1942) demonstrated the formation of reticulin by Schwann cells in vitro. They found that the growth of the cells sometimes left such fibres behind, with no cell connection. It therefore seems worth investigating whether there is any histological evidence of reticulin formation (other than in blood vessels) in the developing nervous system. Silver impregnation methods do not demonstrate the presence of reticulin. The possibility that parts of glial processes may become disconnected from their parent cell and somehow become incorporated in the myelin sheath (perhaps as part of the lipo-protein neuro-keratin), might be investigated further. Windrum, Kent and Eastoe (1955) showed that reticulin had a fatty acid incorporated in its structure.

I also wish to stress that I have considered only the purely structural nature of the glial background. Recent work (Hess,1953) has suggested that there is a ground substance in the spaces between neuroglial fibres, axon terminations, and dendrites. In the developing central nervous system (Hess, 1955) this ground substance makes its appearance at the same stage of development in all parts of the brain. After studying 32 guinea pigs, Hess concluded that the ground substance appeared at the 43rd. - 45th. day of foetal life. The relationship of this ground substance to myelination has not yet been studied.

2 Lipid changes during myelination

I wish now to discuss some of the histological features of the lipid droplets to justify the interpretation that they are a product of glial cells and represent a stage in the formation of the myelin sheath.

The possibility must first be excluded that the fine lipid droplets and the larger lipid deposits, described in the last chapter, are artifacts.

We saw (p. 37) that the occasional occurrence of fine granular deposits on the sections is a technical objection to the use of sudan black. For the present work, the sudan black was always filtered before use, and unstained areas were free of any deposits. If a section showing the large sudanophilic deposits was decolourised in 70% alcohol, and then re-stained with sudan black, the deposits reappeared in the same positions. This was judged by marking the sections. The regularity and predictability of their incidence in certain regions of a section and at certain age groups, was also significant. Droplets are seen in both human and animal material, although in the animal material the larger aggregations are not seen. I therefore regard the presence of these droplets as a real indication of lipid changes.

In the human material, the lipids which I regard as being related to myelin are visible in two forms. The earliest to appear are the fine droplets. These are followed later by larger aggregates. The earliest chiasma examined

was from a 23 weeks foetus, and it must be recorded that at this stage of development isolated myelin sheaths are already present, as well as the lipid droplets. I cannot therefore assert that, in man, lipid droplets invariably appear before axons are myelinated, although the anterior third of the optic nerve of a 20 weeks foetus shows no droplets. It is possible, however, that a different state exists in the chiasmal end of the nerve, because it has been shown that myelination proceeds down the nerve from the chiasmal end. (Table 5). I was unable to obtain material of the chiasma and pyramids of an earlier foetal age to decide the relationship in time between the lipid droplets and the myelin sheaths. The corpus callosum of a 36 weeks foetus also shows the presence of fine droplets and myelinated fibres.

It is in these early stages of myelination that the function of the glial network is apparent. Fine lipid droplets occur along the fibres of the network, which also seems to support the isolated myelinating nerve fibres. (Figs. 11, 17, 30). Is there any evidence, at this stage, of development, that specific cells are associated with myelinating axons ? It is not clear from my histological preparations that the numerous fine droplets in the early myelinating period originate from cells. Some of the larger aggregations, however, clearly have a cellular origin. (Fig. 18) The cells appear to be oligodendroglia, from their shape and from their relationship to neighbouring cells, for they lie

in long chains. There are two possible explanations for the fact that not all the lipid deposits are intra cellular. The first is that disintegration of these cells has occurred after the formation of lipid destined to be incorporated into the myelin of the sheath. The second is that post-mortem autolysis has occurred. Oligodendroglia are known to undergo rapid autolysis, which explains the difficulty of histochemical research on them.

In the animal material, lipid droplets are both intra- and extra- cellular. As in the human material, the cells concerned are glial. The droplets are confined to areas containing myelinating fibres. An apparent exception to this is the presence of droplets in the molecular layer of the cerebellum, where Weigert myelin preparations demonstrate no myelinated fibres (Fig. 26). A sudan black preparations, however, demonstrates a lipid component of the molecular layer in both human and animal material. (Fig. 27). This lipid is presumably not stable and is lost during preparation for the other types of myelin stain.

Examination of the animal material convinces me that lipid droplets appear before myelinated fibres. I conclude that during myelination in man and animals the glial cells show an intense lipid activity, which is absent when myelination is completed. This is not merely a coincidence, if we consider that there is also evidence that axons actually pass through glial cells (p. 68)

The results now presented are therefore an extension of observations made by the workers mentioned in chapter 1 particularly Morrison (1931), Linell and Tom (1931) and Alpers and Haymaker (1934). I have tried to obviate the sources of the criticisms I made on their work.

The results should be compared with observations of Roback and Scherer (1934) on the role of oligodendroglia in myelination. They considered that the gliosis associated with myelin formation was at its maximum in the pyramids in the olivary region during the 7th. month of foetal life, and ceased in the 2nd. month. This gliosis occurred therefore at a period comparable to my estimation of glial lipid activity.

There is biochemical evidence that lipid metabolism varies considerably in foetal and adult life. Srere, Chaikoff, Treitman and Burstein (1950) showed that cholesterol synthesis proceeded extremely rapidly in baby rat brain, whereas adult rat brain apparently synthesised no cholesterol at all. They studied the metabolism of cholesterol in the central nervous system by using acetate labelled with carbon 14. This finding could mean that the cholesterol originally deposited in the myelin sheath, in the central nervous system, remains there inactive during life. Cholesterol perhaps functions in the myelin sheath as an insulating substance by reason of its high dielectric constant. If it is no more than an insulator, then its inactivity is explicable.

There is an indication here of a problem for future investigation, for I have been unable to find if peripheral nerve synthesises cholesterol in adult life. This should be investigated, because of the difference in regeneration processes in peripheral and central nervous system.

Other problems could be investigated by co-operation between histologists and biochemists. The chemical constitution of myelin is gradually being explained, and should be related to its staining properties. We know that the myelin lipids increase in quantity during maturation of the central nervous system. Staining methods indicate no difference between adult and infant myelin. Is the composition of the individual myelin sheath altering, or is the increase in myelin lipids explained by the increasing number of myelinated axons ? This question may be answered as histochemical methods become more reliable. Such methods may also be used to indicate the positions of important lipid groups in the myelin, and their position could then be studied by diffraction or electron microscopy.

3 Morphology of the developing myelin sheath

The developing sheaths are recognisable by their beaded, nodular, appearance. (Figs. 30 - 33). A comparable appearance is seen in developing peripheral nerve. The sciatic nerve fibres show the Schwann cells enveloping the myelinating axon at regular intervals. (Fig. 34). In figure 32 we can see the developing sheath in all its stages except the final regular, tubular, form. The finer fibres have smaller nodules along their course, and the nodules increase in size with the increasing diameter of the sheath. Do we see here the picture which led to the enunciation of the erroneous cell-chain hypothesis of nerve fibre formation? I have remarked previously that I think axons traverse glial cells. If this is so, the appearance of axons passing through cells may have led others to think that the nerve fibre was being formed by the cell-chains.

SUMMARY

1 In the central nervous system, fully developed axons of a diameter greater than 1 μ are covered with a myelin sheath. Biochemical evidence shows that myelin is a mixture of fatty substances, of which the most important are cholesterol, sphingomyelin, cerebrosides, and lipoprotein. It is possible that finer axons also have a lipid covering.

2 The present study is an investigation into the cytology of myelin production in the central nervous system. This work is undertaken because it is uncertain which cells of the central nervous system are responsible for myelin production, and little histological work has been done with human or animal material, with the specific purpose of answering this question. Such work has provided some evidence that oligodendroglia are closely associated with myelin production.

3 The method of study is to investigate cellular activity, with special reference to fatty change in certain regions of human and animal central nervous system, during the period of myelination in these regions.

4 Of the many histological methods available for the demonstration of myelin or its constituents, staining with sudan black is considered to be the most useful. Sudan black is a specific stain for lipids as a group of substances, and it is used on formalin fixed frozen sections to reveal any cellular lipid changes occurring during myelination of axons.

5 Results are presented which show that during myelination, there is an intense lipid activity in glial cells surrounding developing axons. Such activity is not present before or after myelination. It is concluded that glial cells deposit some of the lipid of the myelin sheath. The possibility is not excluded that nerve cell metabolism may also modify the axon sheath.

6 Some problems for further investigation are suggested. There is particular need for a detailed investigation into the staining properties of myelin to determine which of its constituents are responsible for the colour reactions in the various staining methods for myelin.

BIBLIOGRAPHY

Alexander, L. (1938) The neurone as studied by micro - incineration. Brain 61 52

Alpers, B.J., & Haymaker, W. (1934) The participation of the neuroglia in the formation of myelin in the prenatal infant brain. Brain 57 195

Andrew, W. (1943) The reticular nature of glia fibres in the cerebrum of the frog and in the higher vertebrates. J. Comp. Neurol. 79 57.

Ashbel, R. & Seligman, A.M. (1949) A new reagent for the histochemical demonstration of active carbonyl groups. Endocrinology 44 565

Baker, J.R. (1946) The histochemical recognition of lipine. Quart. J. Micr. Sci. 87 441

Barnard, J. & Carpenter, W. (1950) Lack of regeneration in the spinal cord of the rat. J. Neurophysiol. 13 223

Berberich, J. (1926) Besteht ein Zusammenhang zwischen Fettbefunden im Zentralnervensystem neugeborener Menschen und der Markscheidenentwickelung ? Klin. Wochenschr. 5(i) 211

Berenbaum, M.C. (1954) Staining of bound lipids. Nature 174 190

Bielschowsky, M., Bumke, O. & Foerster, O. (1935) Handbuch der Neurologie, vol. i. Berlin, Springer.

Boll, F. (1874) Die Histiologie und Histiogenese der nervösen Centralorgane. Archiv f Psychiatrie. 4 1.

Brantë, G. (1949) Studies on lipids in the nervous system, with special reference to quantitative chemical determination and topical distribution. Acta Physiol. Scand. 18 (supp. 63)

Breemen, V.L. van (1954) Structure of neuroglial cells as observed with the electron microscope (Abstract). Anat. Rec. 118 438

Brodal, A. & Harrison R.G. (1948) Observations on the chemical composition of myelin, and the smallest size of myelinated nerve fibres in the central nervous system. Quart. J. Micr. Sci. 89 89

Brown, J.O. & McCouch, G.P. (1947) Abortive regeneration of the transected spinal cord. J. Comp. Neurol. 87 131

Bruesch, S.R. & Arey, L.B. (1942) The number of myelinated and unmyelinated nerve fibres in the optic nerve of vertebrates. J. Comp. Neurol. 77 631

Cain, A.J. (1947) Demonstration of lipine in the Golgi apparatus of gut cells of Glossiphonia. Quart. J. Micr. Sci. 88 151

- (1947) The use of Nile blue in the examination of lipoids. Quart. J. Micr. Sci. 88 383

- (1948) A further note on Nile blue. Quart. J.Micr. Sci. 89 429

- (1950) The histochemistry of lipoids in animals. Biol. Rev. (Camb. Phil.Soc.) 25 73

Cajal, Ramon Y. (1909-1911) Histologie du Systeme Nerveux. (French edition, translated by L.Azoulay. 2 vol. Paris)

Chang, Min Chueh (1936) A formol thionin method for the fixation and staining of nerve cells and fibre tracts. Anat. Rec. 65 437

Davidoff, L.M. & Ransohoff, J. (1948) Absence of spinal cord regeneration in the cat. J.Neurophysiol. 11 9

Davis, F.A. (1940) Primary tumors of the optic nerve. Arch. Ophthal. (Chicago) 23 735

Deuel, H.J. (1951) The Lipids. Interscience Publishers, Inc. New York.

Eggleton, W.G.E. (1940) The zinc and copper content of the organs and tissues of Chinese subjects. Biochem. J. 34 991

Feyrter, F. (1936) Uber ein sehr einfaches Verfahren der Markscheidenfarbung, zugleich eine neue Art der Farberei. Virchow's Archiv. 296 645

Finean, J.B. (1954) The effects of osmium tetroxide fixation on the structure of myelin in sciatic nerve. Exper. Cell Res. 6 283

Folch, J. (1942) Brain cephalin, a mixture of phosphatides. J. Biol. Chem. 146 35.

Folch, J. (1952) Chemistry of Phosphatides. Phosphorus Metabolism, vol. ii. Baltimore, Johns Hopkins Press.

Folch, J. & Le Baron, F.N. (1951) A trypsin resistant, lipide-protein complex isolated from brain white matter protein. Fed. Proc. 10 183

Folch, J. & Sperry, W. (1948) Chemistry of the lipids. Ann. Rev. Biochem. 17 147

Geren, B.B. (1954) The formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. Exp. Cell Res. 7 558

Gomori, G. (1952) Microscopic histochemistry. Univ. Chicago Press, Chicago.

Govan, A.D.T. (1944) Fat staining by Sudan dyes suspended in watery media. J.Path.& Bact. 56 262

Halliday, N. (1939) The effect of formalin fixation on liver lipids. J.Biol. Chem. 129 65

Hess, A. (1953) The ground substance of the central nervous system revealed by histochemical staining. J. Comp. Neurol. 98 69

- (1955) The ground substance of the developing central nervous system. J.Comp.Neurol. 102 65

- & Lansing A. (1953) The fine structure of peripheral nerve fibres. Anat. Rec. 117 175

Hoerr, N.L. (1936) Histological studies on lipins. Anat. Rec. 66 149

Hogue, M.J. (1947) Human foetal brain cells in tissue culture; their identification and motility. J.Exp.Zool.106 85

- (1953) A study of adult human brain cells grown in tissue culture. Am. J. Anat. 93 397

Holmgren, H. & Rexed, B. (1946) Metachromatic staining of the Schwann cells in nerve regeneration. Acta Anatomica 2 287

Hotchkiss, R. (1948) A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem. 16 131

Hurst, E.W. (1953) The demyelinating diseases of man and animals. Proc. Roy. Soc. Med. 46 887

Jastrowitz, M. (1872) Studien über die Encephalitis und Myelitis der ersten Kindesalters. Arch. f. Psychiat. 3 162

Jeanloz, R. (1950) Hotchkiss reaction and Structure of polysaccharides. Science 111 289

Johnson, A.C., McNabb, A.R. & Rossiter, R.J. (1948a) Lipids of normal brain. Biochem. J. 43 573

- - - (1948b) Lipids of peripheral nerve. Biochem. J. 43 578

- - - (1949a) Concentration of lipids in the brain of infants and adults. Biochem. J. 44 494

- - - (1949b) Chemical studies of peripheral nerve during Wallerian degeneration. 1 Lipids. Biochem. J. 45 500

- - - (1950) Chemistry of Wallerian degeneration. Arch. Neurol. & Psychiat. 64 105

Kaucher, M., Galbraith, H., Button, V. & Williams, H. (1943) The distribution of lipids in animal tissues. Arch. Biochem. 3 203

Kaufmann, C. & Lehmann, E. (1926) Sind die in der histologischen Technik gebräuchlichen Fettdifferenzierungs methoden spezifisch ? Virchow's Archiv. 261 623

Keilig, I. (1944) Uber Spezifitätsbreite und Grundlagen der Markscheidenfärbungen. Virchow's Archiv. 312 405

Kimmelstiel,P. (1929) Uber den Einfluss der Formalin - fixierung von Organen auf die Extrahirbarkeit der Lipoide. Z.physiol. Chem. 184 143

Koch, W. & Koch, M.L. (1913) The chemical differentiation of the brain of the albino rat during growth. J. Biol. Chem. 15 423

Kühne, W. & Chittenden, R.H. (1890) Uber das Neurokeratin. Z. Biol. 26 291

Langworthy, O.R. (1933) Development of behaviour patterns and myelinisation of the nervous system in the human foetus and infant. Contr. Embryol. Carneg. Inst. 34 1

Leach, E.H. (1938) Fat staining with Sudan black B. J.Path. & Bact. 47 635

Linell, E. & Tom, M.I. (1931) Postnatal development of the oligodendroglia cell. Anat. Rec. 48 suppl.45. 27

Lison, L. & Dagnelie, J. (1935) Methodes nouvelles de coloration de la Myeline. Bull. d'histol. appliq. a la physiol. 12 85

Lison, L. (1936) Histochimie Animale. Paris.

Little, K. & Windrum, G.M. (1954) A lipid component of reticulin. Nature 174 789

Logan, J.E., Mannell, W.A. & Rossiter, R.J. (1952) Chemical studies of peripheral nerve during Wallerian degeneration. 3. Nucleic acids and other protein bound phosphorus compounds. Biochem.J. 51 482

Lucas Keene, M.F. & Hewer, E.E. (1931) Some observations on myelination in the human central nervous system. J. Anat., Lond. 66 1.

Lumsden, C.E. (1951) Fundamental problems in the pathology of multiple sclerosis and allied demyelinating diseases. Brit. Med. J. (i) 1035

- & Pomerat, C.M. (1951) Normal oligo dendrocytes in tissue culture. Exper. Cell Res. 2 103

MacArthur, C.G. & Doisy, E.A. (1919) Quantitative chemical changes in the human brain during growth. J. Comp. Neurol. 30 445

McManus, J.F. (1948) Histological and histochemical uses of periodic acid. Stain Technology 23 99

Menschik, Z. (1953) Nile blue histochemical method for phospholipids. Stain Technology 28 13

Michaelis, L. & Granick, S. (1945) Metachromasy of basic dyestuffs. J. Amer. Chem. Soc. 67 1212

Morrison, L.R. (1931) The role of oligodendroglia in myelinogenesis. Trans. Amer. Neurol. Assoc. 444

Murray, M.R. & Stout, A.P. (1942) Demonstration of the formation of reticulin by Schwannian cells in vitro. Amer. J.Path. 18 585

Nageotte, J. (1910) Les etrainglements de Ranvier et les espaces interannulaire des fibres nerveuses a myeline. C.R.Assn. Anat. 12 30

- (1911) Betrachtungen über den tatsächlichen Bau und die künstlich hervorgerufenen Deformationen der markhaltigen Nervenfaser. Arch.f.mikr.Anat. 77 245

Noback, C.R. & Montagna, W. (1952) Histochemical studies of the myelin sheath and its fragmentation products during Wallerian degeneration. J.Comp. Neurol 97 211

Palade, G. (1952) A study of fixation for electron microscopy. J.Exp.Med. 95 285

Penney, J.R. & Balfour, B.M. (1949) The effect of vitamin C on mucopolysaccharide production in wound healing. J.Path. & Bact. 61(ii) 171.

Pischinger, A. (1943) Der formaldehyd als Ursache für die besonderen Erscheinungen bei der Einschlussfärhung. Z. f. Mikr. Anat. Forsch. 53 46

Pomerat, C.M. (1955) Dynamic Neuropathology. J.Neuropath. & Exp. Neurol. 14 28

Randall, L.O. (1938) Chemical Topography of the Brain. J. Biol. Chem. 124 481

Roback, H.N. & Scherer, H.J. (1935) Über die feinere Morphologie des frühkindlichen Gehirns unter besondere Berücksichtigung der Gliaentwickelung. Virchow's Archiv. 294 365

Robertson, J.D. (1955) The ultrastructure of adult vertebrate peripheral myelinated nerve fibres in relation to myelinogenesis. J.Biophysic. & Biochem. Cytol. 1 271

Romanes, G.J. (1947) The prenatal medullation of the sheep's nervous system. J.Anat., Lond. 81 64

Sattler, C.H. (1915) Uber die Markscheidenentwickelung im Tractus Opticus, Chiasma, und Nervus Opticus. Archiv. f. Ophthal. (v.Graefe) 90 271

Schmidt, G., Benotti, J., Hershmann, B., Thannhauser, S.J. (1946) A micromethod for the quantitative partition of phospholipid mixtures into monoaminophosphatides and sphingomyelin. J.Biol.Chem. 166 505

Schmitt, F.O. & Bear, R.S. (1939) The ultrastructure of the nerve axon sheath. Biol.Rev. 14 27

- - & Palmer, K.J. (1941) X-ray diffraction studies on the structure of the nerve myelin sheath. J.Cell. & Comp.Physiol. 18 31

Schulz, A. (1924) Eine methode der mikrochemischen cholesterinnachweises am Gewebsschnitt. Zbl. allg. Path.path.Anat. 35 314

Schuwirth, K. (1940) Uber die Lipoide des Menschlichen Gehirns während der Entwickelung. Z. für Physiol. Chem. 263 25

- (1943) Die Lipoide des Menschlichen Rückenmarks. Z. für Physiol. Chem. 278 1

Schwartz, P. (1924) Erkrankungen des Zentralnervensystems nach traumatischer Geburtsschädigung. Z. f.d. ges. Neurol. u. Psychiatrie 90 263

Setterfield, H.R. & Sutton T.S. (1935) The use of polarised light in the study of myelin degeneration. Anat. Rec. 61 397

Smith, J. Lorrain (1907) On the simultaneous staining of neutral fat and fatty acids by oxazine dyes. J. Path. & Bact. 12 1

- & Mair, W. (1909) An investigation of the principle underlying Weigert's method of staining medullated nerve. J. Path. & Bact. 13 14

- - (1912-13) Development of lipoids in brain of puppy. J. Path. & Bact. 17 123

- - Lipids of the white and grey matter of the human brain at different ages. J.Path. & Bact. 17 418

Srere, P.A., Chaikoff, I.L., Treitman, S.S. & Burstein, L.S. (1950) The extrahepatic synthesis of cholesterol. J. Biol. Chem. 182 629

Sugar, O. & Gerard, R.W. (1940) Spinal cord regeneration in the rat. J. Neurophysiol. 3 1

Sylven, B. (1941) Uber das Vorkommen von hochmolekularen Esterschwefelsaüren im Granulationsgewebe und bei der Epithelregeneration. Acta Chirurg.Scand. 86 (supp.66).

Thannhauser, S.J., Benotti, J., Walcott A. & Reinstein, H. (1939) The lecithin, cephalin and sphingomyelin content of normal human organs. J. Biol. Chem. 129 717

Thannhauser, S.J. & Boncoddo, N.F. (1948) The chemical nature of the fatty acids of brain and spleen sphingomyelin. The occurrence of saturated and unsaturated sphingosines in the sphingomyelin molecule. J.Biol.Chem. 172 141

Thudichum, J.L.W. A treatise on the chemical constitution of the brain. London 1884

Tuthill, C.R. (1938) Fat in the infant brain in relation to myelin, blood vessels, and glia. Arch.Path. 25 336

Virchow, R. (1867) Zur pathologischen Anatomie des Gehirns. Virchow's Archiv. 38 129

Weigert, K. (1895) Quoted by Glees, P. (1955) Neuroglia Oxford.

Weil, A. (1929) The influence of formalin fixation on the lipoids of the central nervous system. J.Biol. Chem. 83 601

Williams, H., Galbraith, H., Kaucher, M., Moyer, E.Z., Richards, A.J. & Macy, I.G. (1945). The effect of growth on the lipid composition of rat tissues. J. Biol. Chem. 161 475

Windrum, G.M., Kent, P.W. & Eastoe J.E. (1955) The constitution of human renal reticulin. Brit.J.Exp.Path. 36 49

Wislocki, G.B. & Singer, M. (1950) The basophilic and metachromatic staining of myelin sheaths and its possible association with a sulfatide. J.Comp.Neurol. 92 71

Wlassak, R. (1898) Die Herkunft des Myelins. Archiv. f. Entwickelungsmechanik 6 453

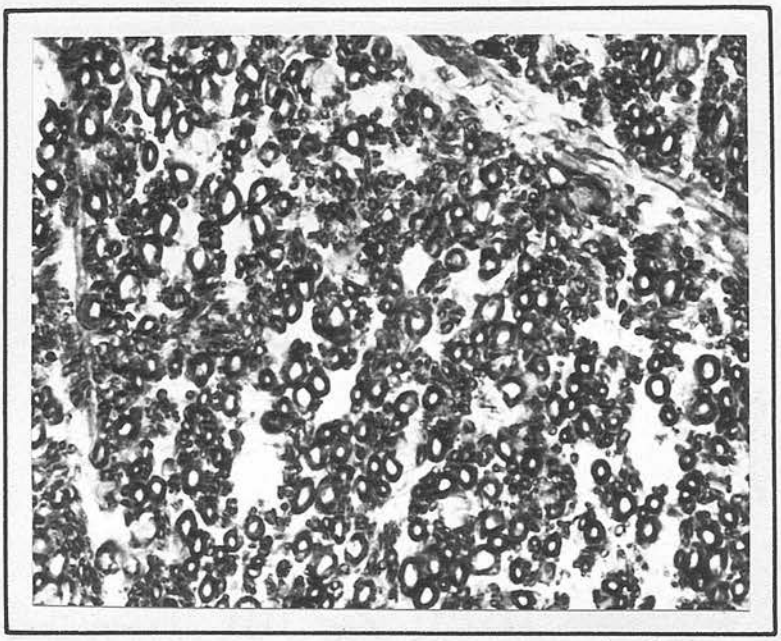
Wohlwill, F. (1921) Zur Frage der sogenannten Encephalitis congenita (Virchow). I. Ueber normale und pathologische Fettkörnchenzellbefunde bei Neugeborenen und Säuglingen. Z.f.d. ges. Neurol. u.Psychiatrie 68 384

Wolman, M. (1950) Staining of lipids by the periodic acid Schiff reaction. Proc.Soc.Exp.Biol.Med. 75 583

- & Greco, J. (1952) The effect of formaldehyde on tissue lipids and on histochemical reactions for carbonyl groups. Stain Technology 27 317

The microscope, microtomes and staining materials for most of this work were provided by the W.H.Ross Foundation for the Prevention of Blindness. The sections were prepared by the author. The photomicrographs were produced by the Photography Department, Department of Pathology, Edinburgh University.

Fig. 1



X 375

Adult human spinal cord. Fixed formalin. Frozen section, stained Sudan black. Shows normal staining of myelin sheaths by Sudan black.

Fig. 2

Adult human spinal cord. Treated with absolute alcohol / chloroform mixture before formalin fixation. Frozen section, stained Sudan black.

No recognisable sheaths remain.

X 375

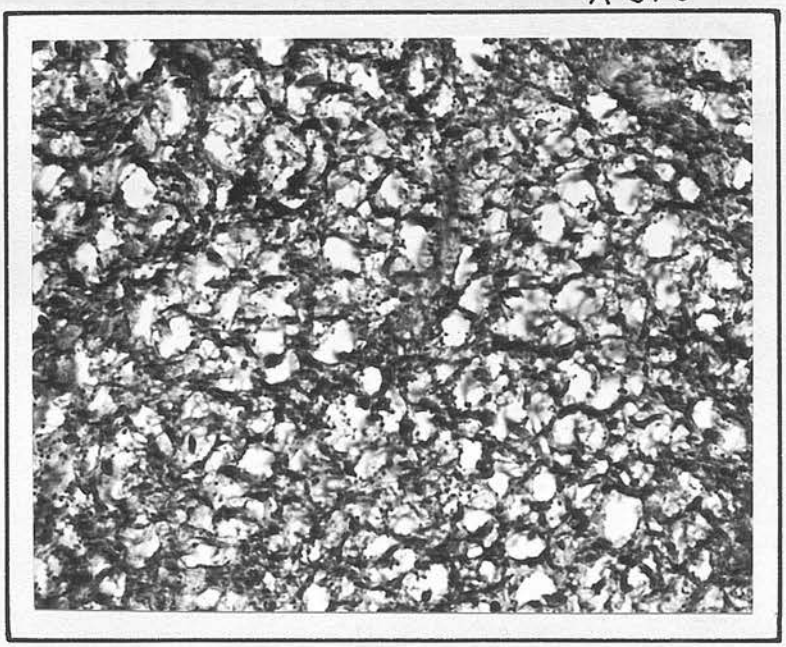
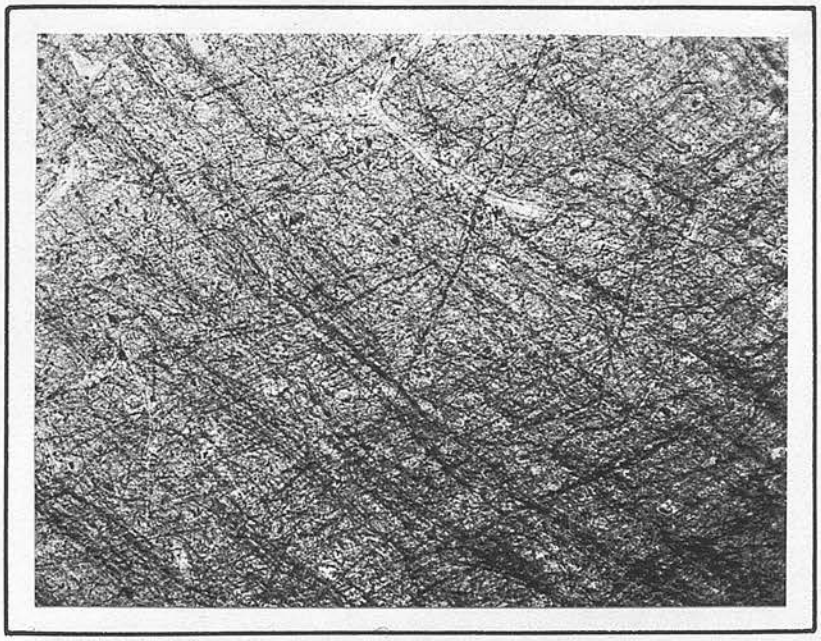


Fig. 3



X100

Adult human cortex, innermost part. Dehydrated through alcohols, and treated with acetone before formalin fixation.

Myelin sheaths still recognisable. Background dirty.

Fig. 4

Adult human spinal cord. Fixed formalin. Frozen sections extracted for 24 hours in absolute alcohol / chloroform mixture after fixation. Stained Sudan black. Sheaths of lipid substance are still visible.

X375

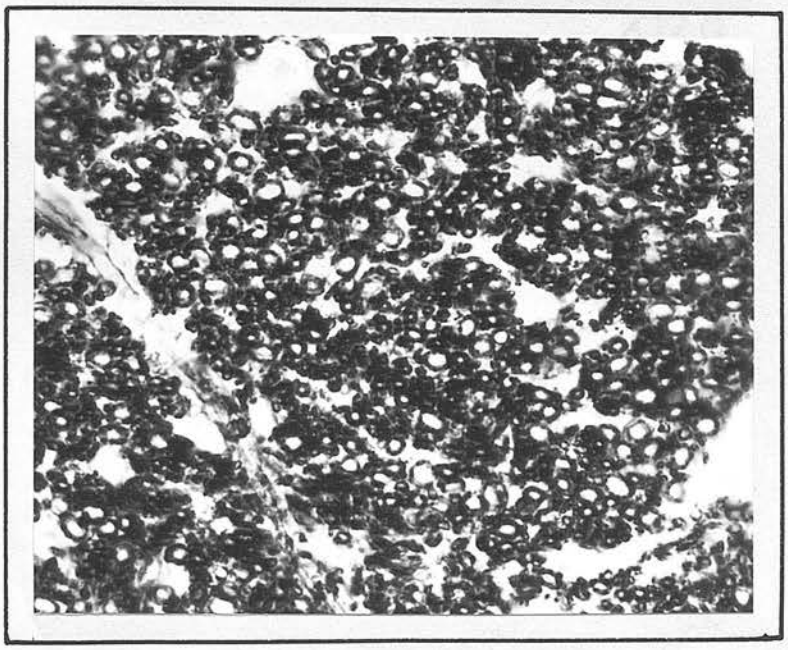
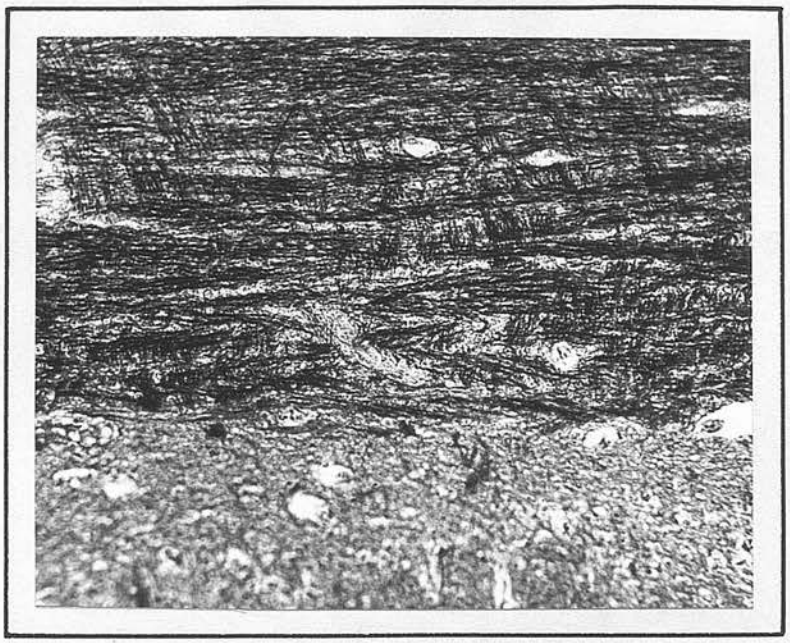


Fig. 5



X120

36 weeks human foetus. Medulla.
Fixed formalin. Frozen section,
stained Sudan black.
Shows bundle of myelinated fibres.

Fig. 6

Adjacent section to that shown in figure 5.
Immersed 24 hours in absolute alcohol/
chloroform mixture after cutting.
No gross change detectable

X 120

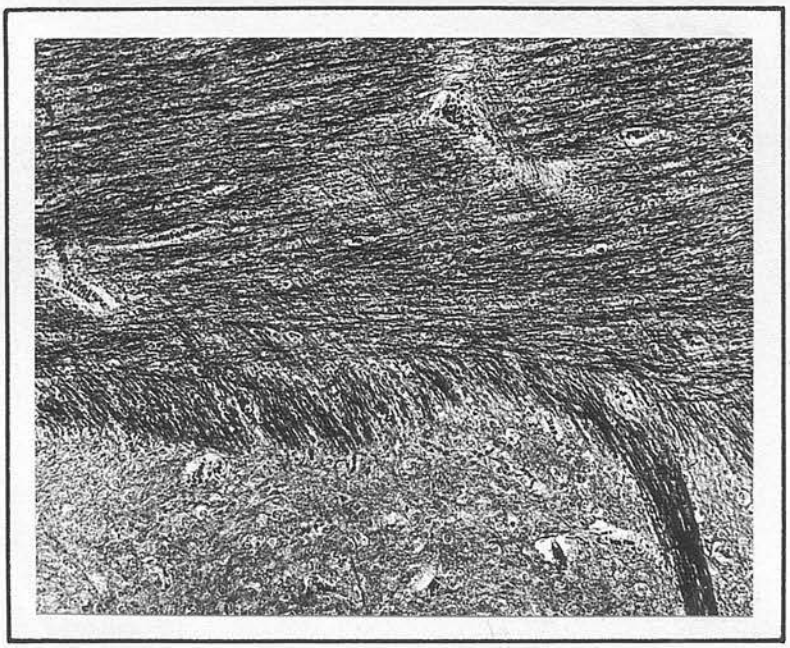
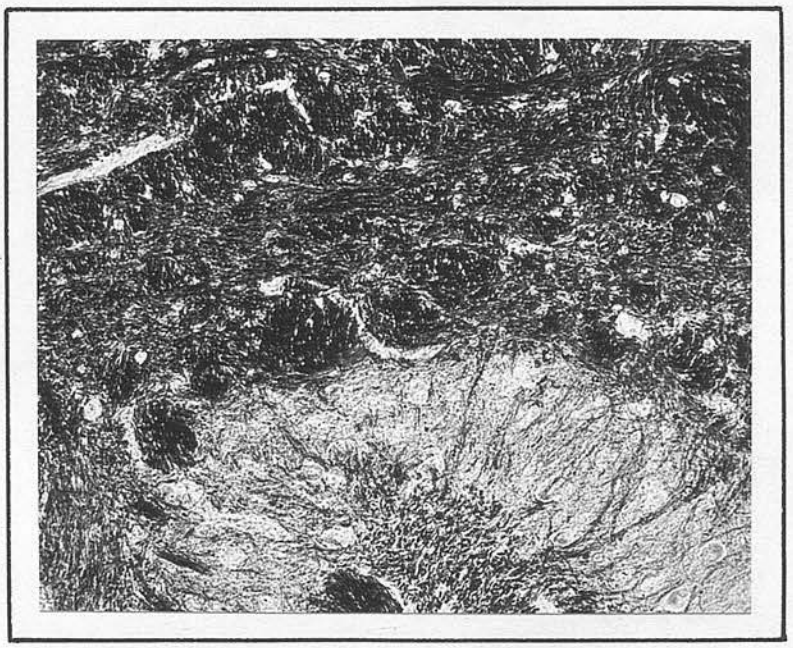


Fig. 7



X120

Adult medulla. Fixed formalin. Frozen section, stained Sudan black.
Shows bundles of myelinated fibres.

Fig. 8

Adjacent section to that shown in figure 7.
Immersed 24 hours in absolute alcohol/
chloroform mixture after cutting.
No gross change detectable

X120

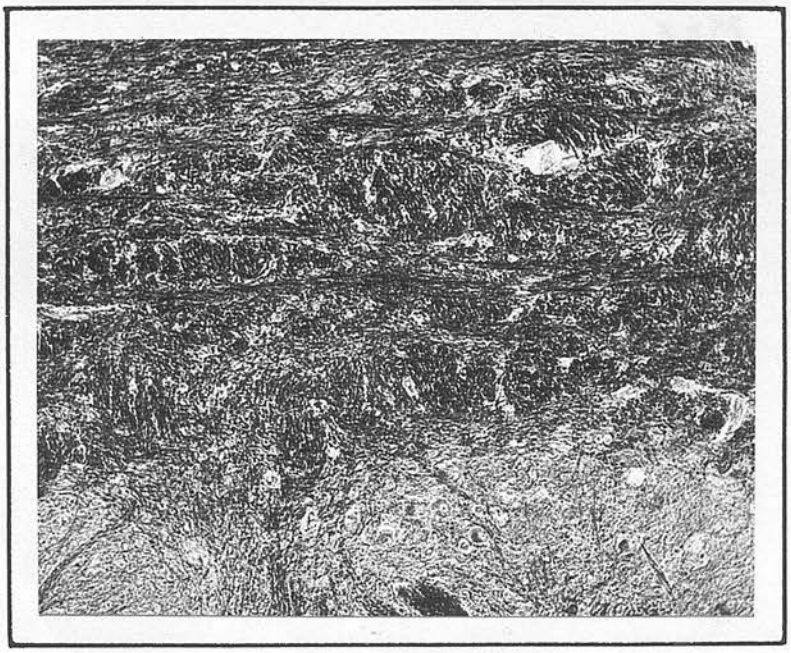
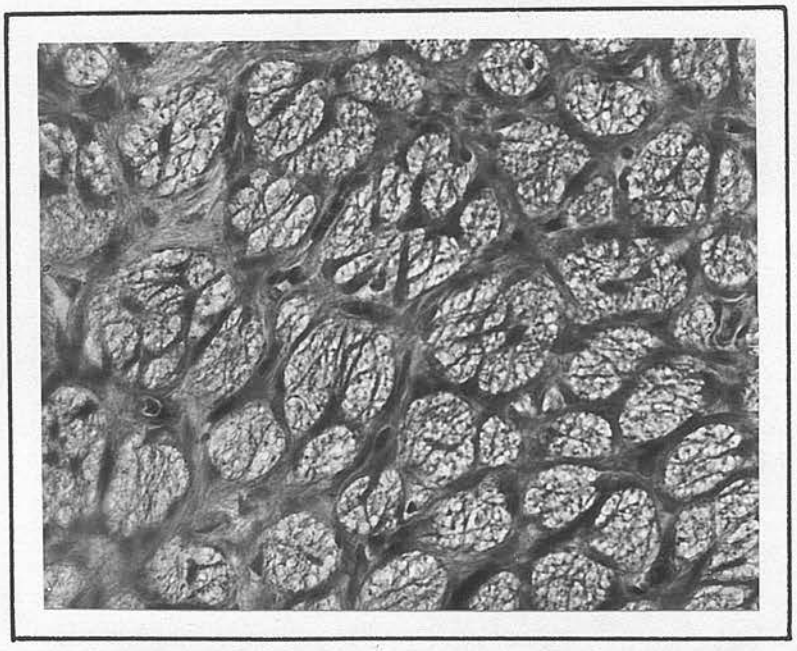


Fig. 9



X375

20 weeks human foetus. Optic nerve, anterior third. Fixed formalin. Frozen section, stained van Gieson. Shows fine network within coarser septa.

Fig. 10

32 weeks human foetus. Optic nerve, posterior third. Fixed formalin Frozen section, stained van Gieson Shows a fine network.

X 375

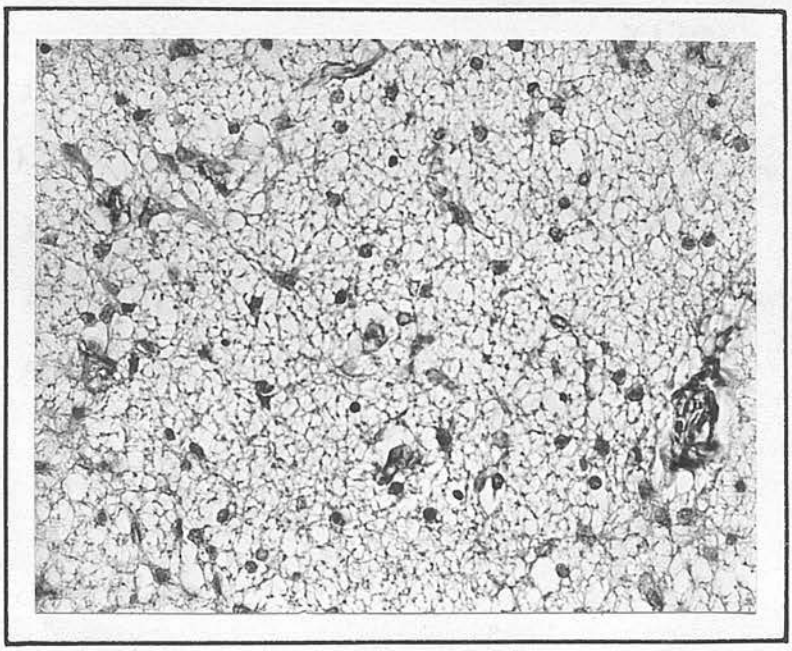


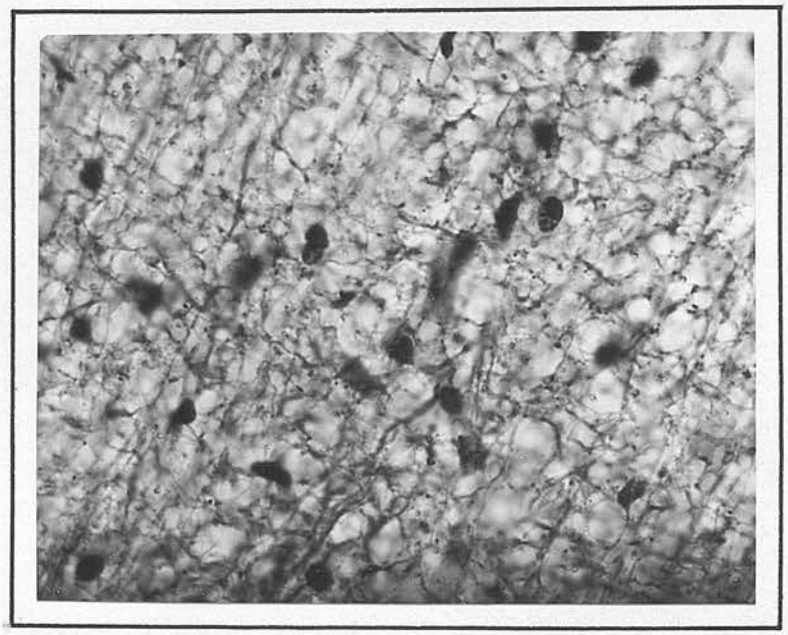
Fig. II

X1200

23 weeks human foetus. Optic chiasma, horizontal section. Formalin fixation, frozen section. Stained Sudan black.

Shows a myelinating fibre in the sudanophilic reticular background.

Fig. 13



X 785

38 weeks foetus, human. Medulla.
Formalin fixation. Frozen sections.
Phosphotungstic acid haematoxylin to
show processes.

Fig. 14

30 weeks human foetus. Medulla.
Formalin fixation, frozen sections.
Stained Sudan black.
Shows numerous lipid deposits.

X 80

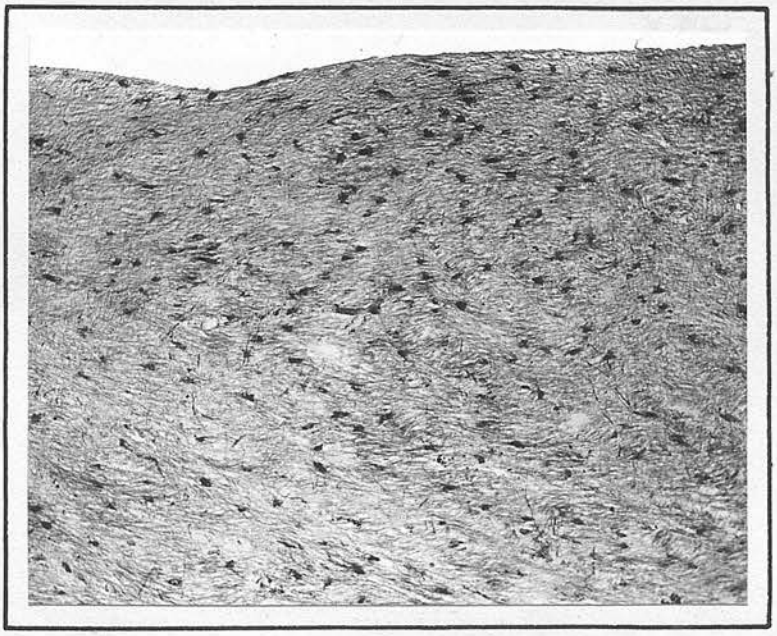
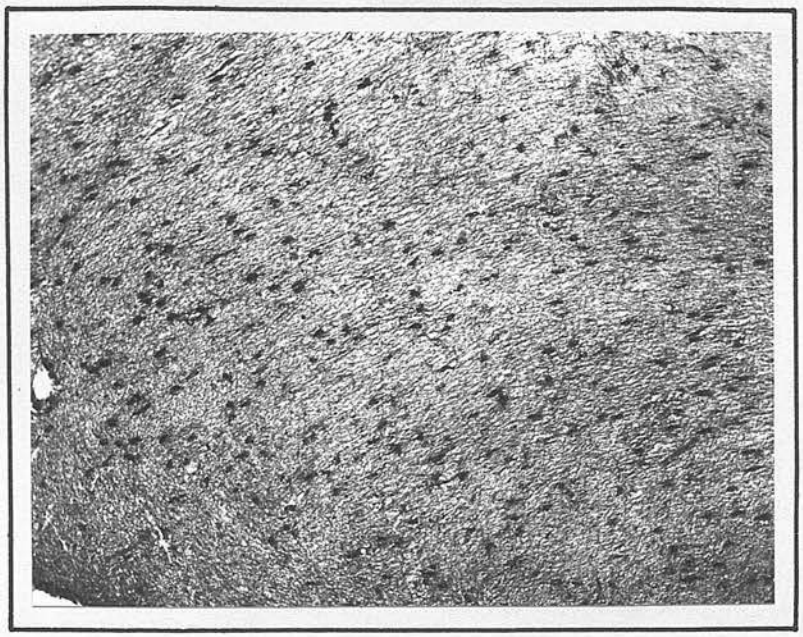


Fig. 15



×80

30 weeks human foetus. Optic chiasma.
Formalin fixation, frozen sections.
Stained Sudan black.
Shows numerous lipid deposits.

Fig. 16

Same section as shown in figure 15
Shows relationship of Sudanophilic
deposits to network in background

X 375

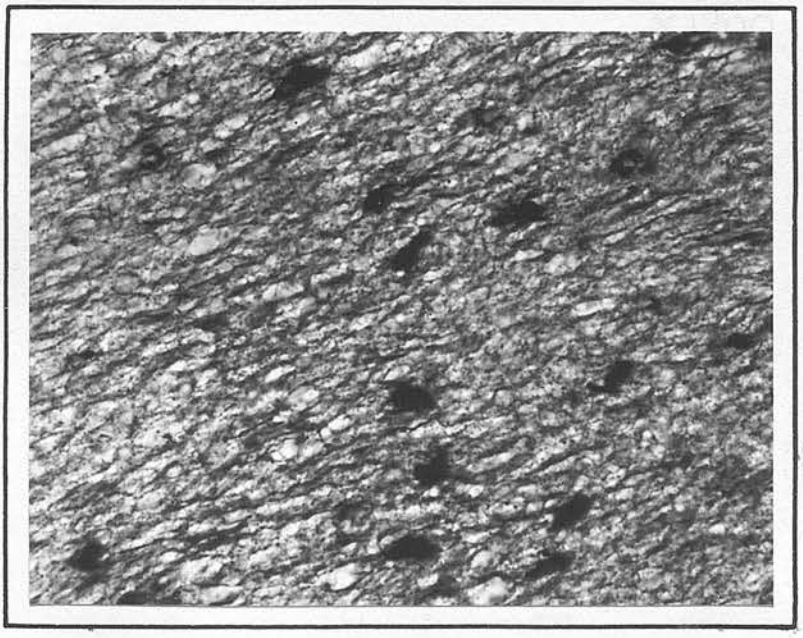


Fig. 17

X 1200

Same section as shown in figure 15
Shows a single lipid deposit, and the numerous fine droplets in the meshes of the network.

Fig. 18

36 weeks human foetus. Optic chiasma.
Formalin fixation, frozen section.
Sudan black, counterstained alum haematoxylin (without blueing).
Shows the relationship of one of the lipid deposits to a cell and nucleus (CN).
X800

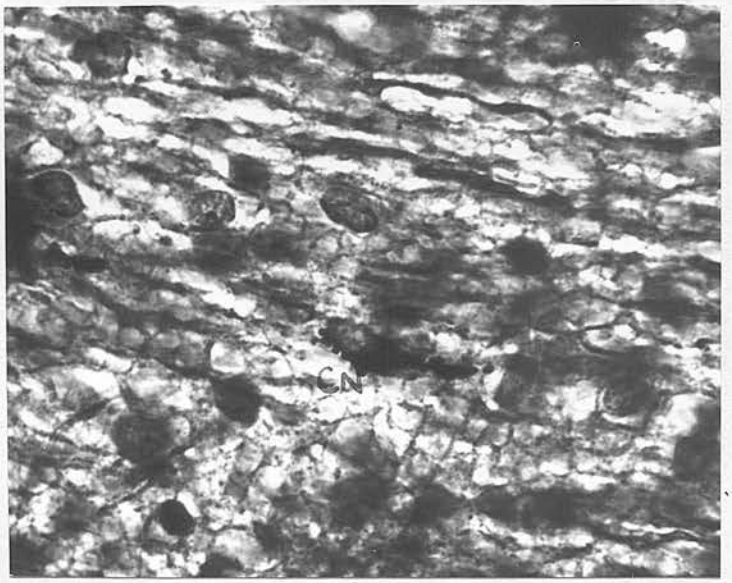
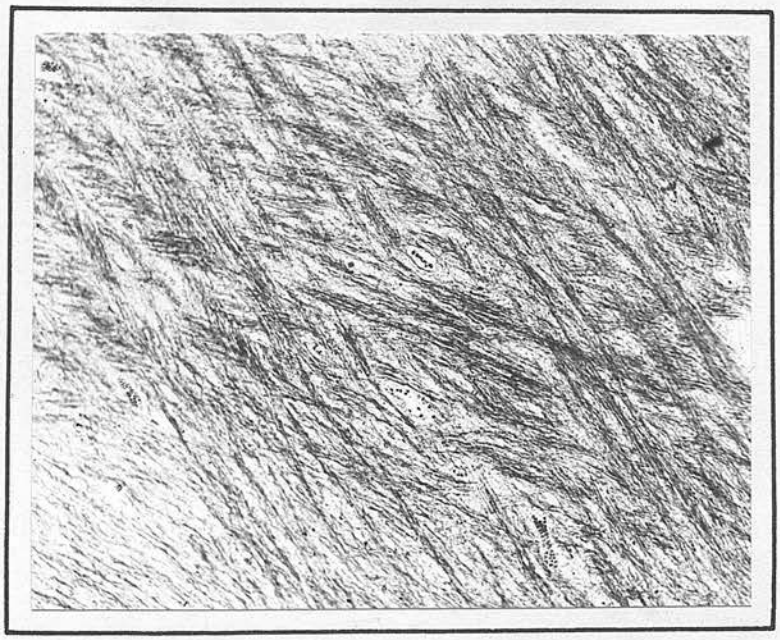


Fig. 19



X100

Full term human foetus. Optic chiasma.
Formalin fixation, frozen sections.
Stained Sudan black.
Fibres are myelinated, and the decussation
is evident. No lipid deposits are now
visible.

Fig. 20

Sheep foetus, C–R length 24 cm. Formalin fixation, frozen section. Stained Sudan black. There is no counterstain, but cell outlines show up faintly. The relationship of lipid droplets to cells and myelinated fibres can be seen.

×480

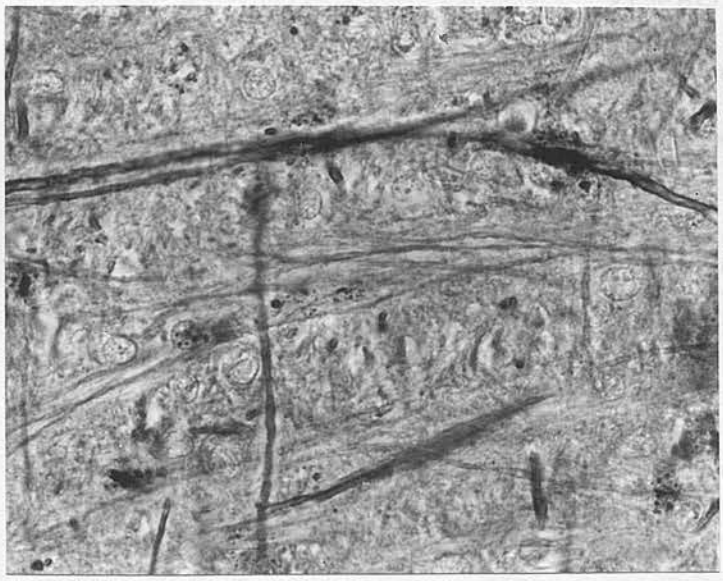
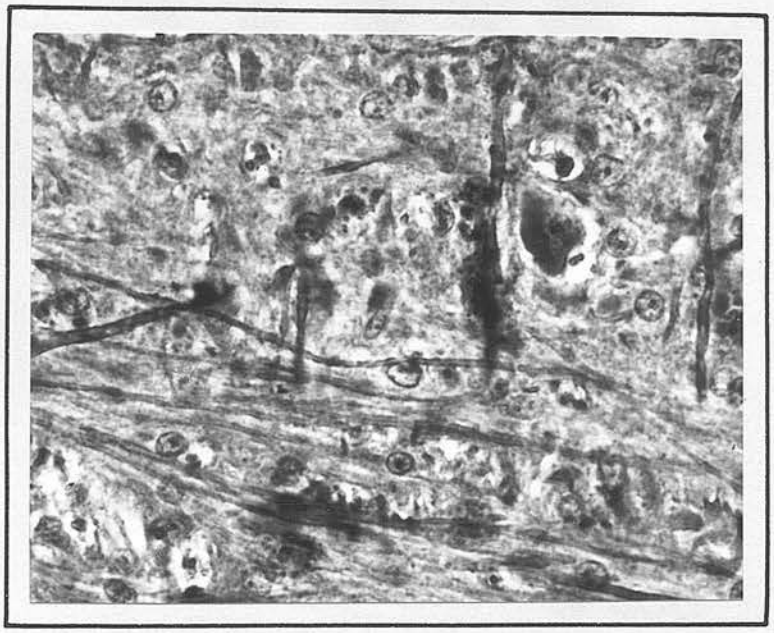


Fig. 21



X480

Sheep fœtus, C-R length 24 cm. Frozen section, stained Sudan black, counterstained alum haematoxylin. This figure should be compared with figure 22, which is a higher power view of part of the field shown above. Together, these two illustrations show how different focussing demonstrates the relationship of cells, droplets, and myelinating fibres.

Fig. 22

Sheep foetus, C–R length 24 cm. Frozen section, stained Sudan black, counterstained alum haematoxylin.

Shows the relationship between cells droplets, and myelinating fibres. Compare with figure 21.

× 800

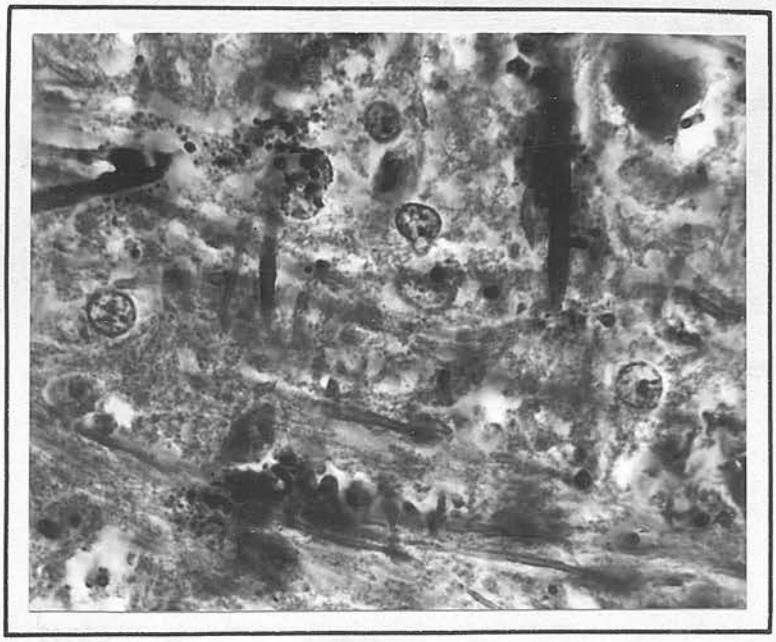
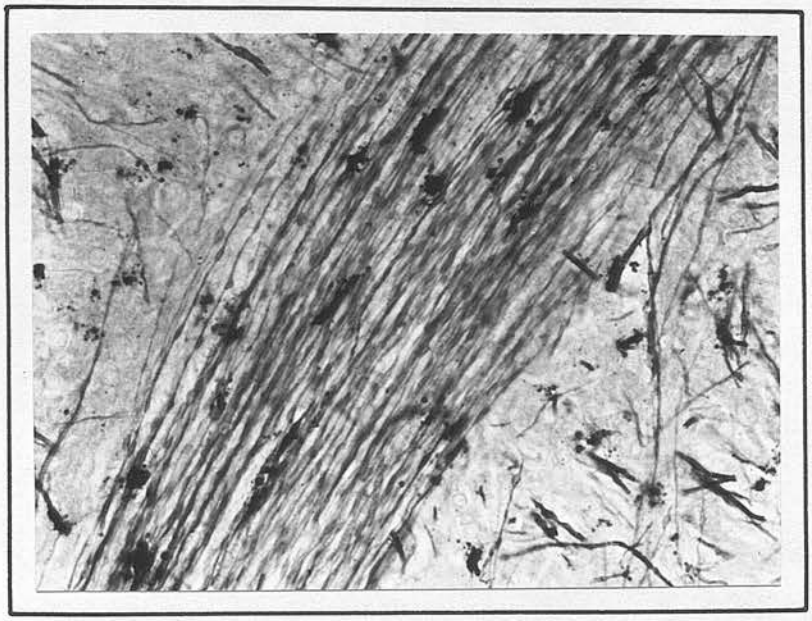


Fig. 23



X300

Sheep foetus, C–R length 22 cm. Formalin fixation, frozen sections. Transverse section through lower border of pons.

Shows part of the root of the 7th nerve. Many fibres are myelinated. Along their course, cells containing lipid are seen. The characteristic nodular appearance of developing fibres is seen at the outer border of the nerve.

Fig. 24

Sheep foetus, C-R length 27 cm. Formalin fixation, frozen sections. Transrerse section through lower border of pons. Shows part of the 7th nerve, before it emerges from the pons. The majority of the fibres now appear to be myelinated. Few lipid cells

X 300

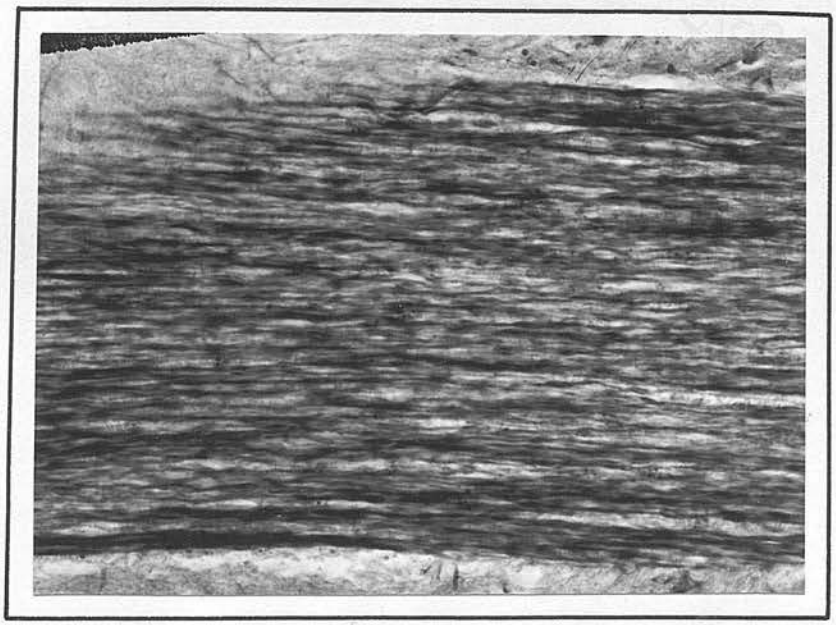


Fig. 25

X100

Sheep foetus, C-R length 24 cm. Formalin fixation, frozen sections. Stained Sudan black. The pattern of a cerebellar folium can be seen. At this stage of development lipid cells are present along the myelinating fibres. They are also present in the molecular region

Fig. 26

Adult human cerebellum. Paraffin sections, stained by Loyez method for myelin. No myelinated fibres are present in the molecular layer. Compare with figure 27 (Section provided by Dept. Neuropathology Edinburgh Univ.)

X50

Fig. 27

×50

Adult human cerebellum. Formalin fixation frozen section, stained Sudan black. Shows a lipid component of the molecular layer. Compare with figure 26

Fig. 28

Human foetus, 38 weeks. Medulla, sagittal section. Formalin – ammonium bromide fixation Frozen section. Hortega's silver carbonate impregnation for oligodendroglia. Shows the chains of oligodendroglia nuclei in the pyramidal bundles

X 100

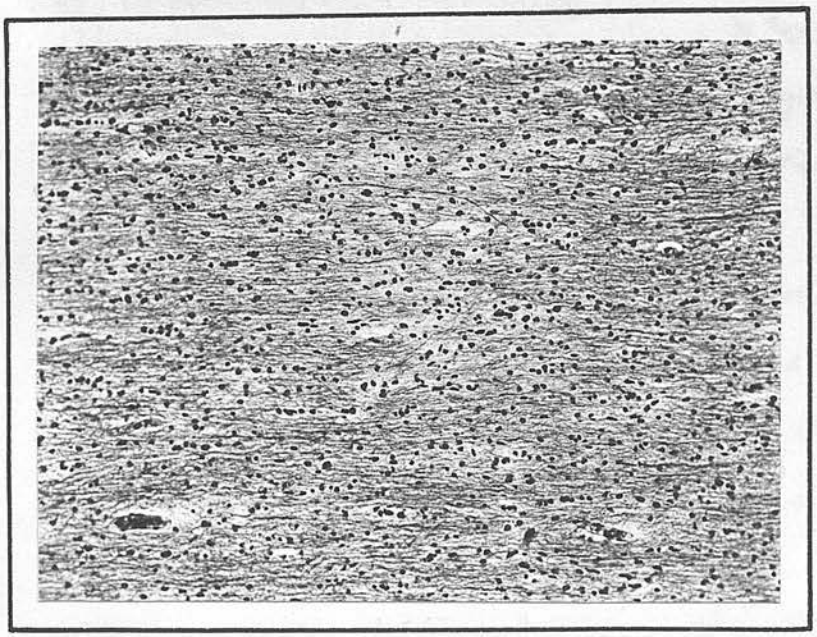
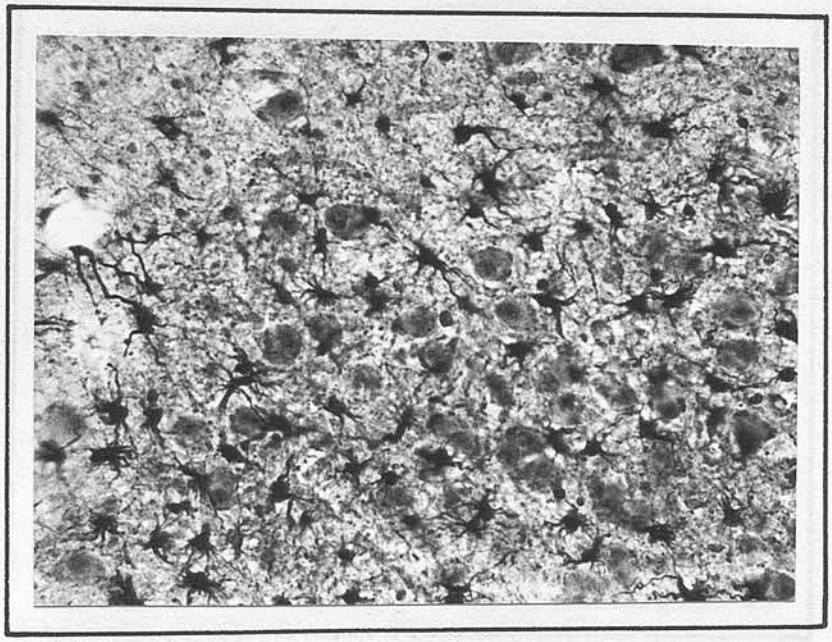


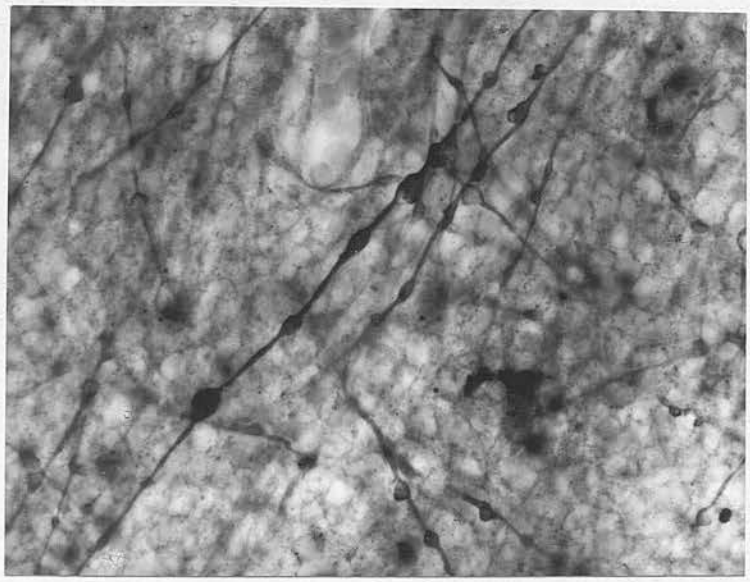
Fig. 29



×300

Human foetus, 38 weeks. Medulla, sagittal section. Formalin – ammonium bromide fixation. Frozen section. Cajal's gold sublimate method for astrocytes. In this section, very few astrocytes were demonstrated between the pyramidal bundles. The illustration shows astrocytes in an adjacent region

Fig. 30



X625

Human foetus, 36 weeks. Optic chiasma. Formalin fixation, frozen sections. Stained Sudan black.

Shows the background network, with lipid droplets, and nodular myelinating fibres.

Fig. 31

Human foetus, 36 weeks. Medulla, sagittal section. Formalin fixation, frozen section, Stained Sudan black. Shows the background network and lipid droplets.

X 625

Fig. 33

Human foetus, 38 weeks. Medulla, sagittal section. Formalin fixation, frozen section. Stained Sudan black. This is part of the section in the previous illustration. There is no counterstain, but fine focussing with the oil immersion lens showed that many fibres appeared to pass through cells

X1200

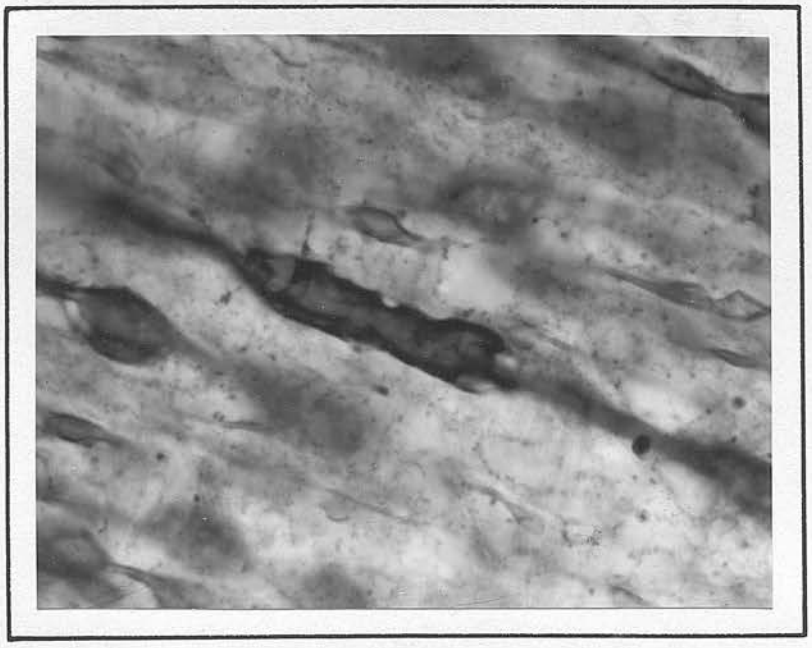
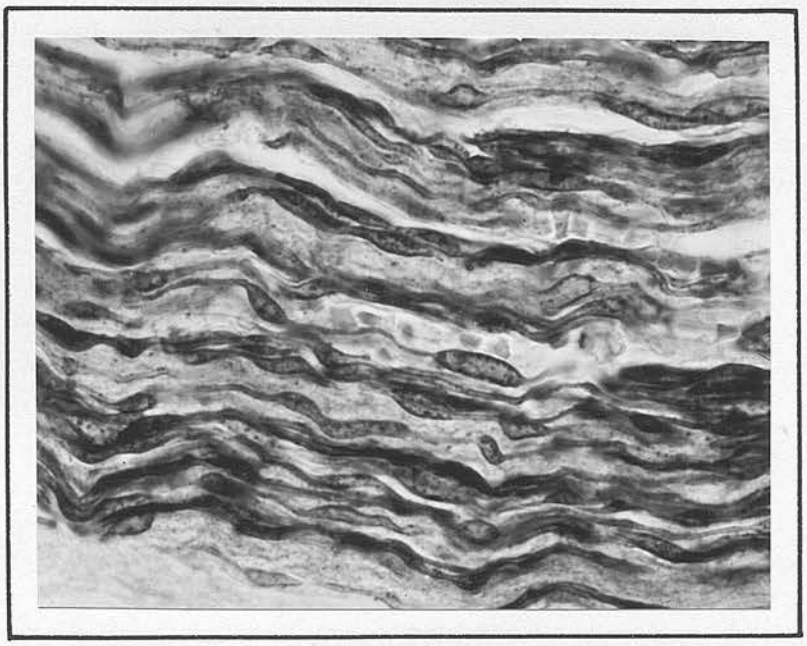


Fig. 34



X 475.

31 weeks foetus, human. Sciatic nerve. Formalin fixation, frozen section. Stained Sudan black. This section of a peripheral nerve is for comparison with the fibres of the central nervous system. The section shows how the Schwann cells are related to myelinating fibres.